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The Effect of Forest Fragmentation on Genetic Diversity, Mating Systems and Effective Population Sizes of Forest Trees in Guanacaste, Costa Rica

by

©

Jonathan Philip Cornelius

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in

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled 'The Effect of Forest Fragmentation on Genetic Diversity, Mating Systems and Effective Population Sizes of Forest Trees in Guanacaste, Costa Rica' submitted by Jonathan Philip Cornelius in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Forest Biology and Management.

Francis C. Yeh

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for Timothy J.B. Boyle

S. Ellen MacDonald

Felix A.H. Sperling

April 16, 2003

For Norma, Paul and Emma

Abstract

This thesis addresses the genetic impact of forest fragmentation on remnant tree populations in Guanacaste, Costa Rica. Chapter One justifies the research in terms of the importance of genetic diversity and possible fragmentation effects. Chapter Two reviews the latter. Chapter Three traces the deforestation history of the study zone and discusses its genetic implications, particularly those of increased forest linearity. Chapters Five and Seven report studies of two native species (Chapters Four and Six describe inheritance, neutrality, linkage of the allozyme markers used). *Anacardium excelsum* gene diversity (H_e) was similar to other tropical woody species. There was no apparent relationship between gene diversity and population size. There was moderate to large subpopulation differentiation overall, but less genetic differentiation within population groups, and there was an apparent relationship between genetic and geographical distances. F_{st}-based migration estimates were around 1 individual generation^{-1} overall, but 2-4 generation^{-1} within groups. Current gene flow to one isolated population (m) was 0.18. Outcrossing estimates (t_m) varied between fragments (mixed mating system), and were significantly positively related to neighbourhood density. Sheltered fragments on watercourses tended to higher flowering and flowering equitability. Growth rate in common garden experiments varied significantly between fragments; it was not significantly related to t_m, but tended to be lower in highly disturbed populations. *Plumeria rubra* H_e was similar to *A. excelsum.* Subpopulation differentiation was low to moderate. F_{st}-based migration estimates were 2-7 migrants generation^{-1}, whilst m to one population was 0.13. Estimated population t_m were not significantly different from 100%. Capsule production was positively related to neighbourhood density. Results suggest differing susceptibilities to fragmentation. Disturbance may reduce *A. excelsum* effective population size due to inbreeding and high fertility variance. Despite smaller population sizes, anemochory, and aggregated populations,

P.rubra retains high variation and little subpopulation differentiation, probably due to its highly mobile pollinator, whilst self-incompatibility precludes outcrossing effects. However, low genetic variation in one population, and density-related limitation of fruiting, suggest the species's resilience has limits. In Chapter Eight, general implications are discussed, with reference to species and forests of the zone, and to genetic and reproductive processes. The mitigation of fragmentation by improved husbandry of pastureland and riparian trees is considered.

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LIST OF ABBREVIATIONS

A	allelic richness
AAT	aspartate aminotransferase
ADH	alcohol dehydrogenase
AP	allelic richness of polymorphic loci
a.s.l.	above sea level
A_e	effective allelic richness
AK	adenylate kinase
d.f.	degrees of freedom
E.C.	enzyme commission
F_e	estimated equilibrium value of inbreeding coefficient for a given outcrossing rate
F_{is}	correlation between uniting gametes relative to subpopulations
F_{it}	correlation between uniting gametes relative to the population as a whole
F_{st}	correlation between two gametes drawn randomly within subpopulations relative to that of two gametes drawn randomly from the population as a whole
Hcda.	Hacienda
H_e	expected heterozygosity, gene diversity
hr	hour
IBD	isolation by distance
J	Shannon's equitability index
LAP	leucine aminopeptidase
m	migration rate
MDH	malate dehydrogenase
N	(1)census population size; (2)north
NDI	neighbourhood density index
N_e	effective population size
OMPG	one migrant per generation
p	(1)allele frequency; (2)probability
P	proportion of loci that are polymorphic
PGD	phosphogluconate dehydrogenase
PGI	glucose-phosphate isomerase
PGM	phosphoglucomutase
q	allele frequency;
r	(1)allele frequency; (2)Pearson's correlation coefficient
R^2	coefficient of determination
s	(1)standard deviation; (2)allele frequency;
$s_{\bar{x}}$	standard error
UGPU	UTP-glucose-1-phosphate uridylyltransferase
W	west

Chapter One

INTRODUCTION

Selection between genetic variants is the logical and practical base of the domestication and genetic improvement of alimentary animals and plants. Consequently, genetic diversity, without which effective selection is impossible, has long been, and remains, basic to human welfare. However, genetic diversity also has a wider importance: the lack of breeding potential and the susceptibility to pests and diseases common in many genetically depauperate domesticates (Smith *et al.*, 1992) are paralleled in depauperate wild species by reduced evolutionary potential and fitness, implying that genetically impoverished populations and species are likely to be more susceptible to extinction (Brook *et al.*, 2002; Frankham, 1998). This connection of genetic diversity to species and, ultimately, community and ecosystem persistence, implies that the value of genetic diversity also must include the wide range of values associated with biodiversity in general, i.e. those encapsulated in the Convention on Biological Diversity (CBD, 1992) as 'intrinsic... ecological, genetic, social, economic, scientific, educational, cultural, recreational and aesthetic values'.

Much of the world's biodiversity is lodged in forests, particularly tropical forests, which are estimated to contain up to 70 per cent of the world's species (Groom, 1994). However, over the last two thousand years or so, the exponential growth of human populations, coupled with the growth of cities, of industrialization, and with the requirements of domesticated animals and plants, has led to widespread destruction and degradation of forested and other natural ecosystems. Approximately half of the world's forest area has been cleared or degraded since the beginning of the Holocene (Groombridge and Jenkins, 2002), with about 30 per cent of the world's land area currently under forest (FAO, 2001). In recent decades, loss has been particularly severe in the tropics, where, in the ten years to 2000 alone, the area of natural forest declined from 1,945 million ha to 1,803 million ha (FAO, 2001).

Complete forest removal leads to loss of whatever unique genetic characteristics were previously present and, concomitantly, contributes to the loss of the set of biodiversity values mentioned above. However, the threat to genetic diversity posed by deforestation may be both more insidious and more pervasive than suggested by the above estimates. Recent studies suggest that only about one-third of remaining world forest is unfragmented (Riitters *et al.* 2000). The ultimate impact of deforestation depends on the permanence of such fragments, which in turn depends on the viability of their plant and animal populations. Such viability is not assured, because small populations are subject to a number of pressures which are absent or of less importance in large populations. When small population effects such as genetic and demographic stochasticity, Allee effects and inherent susceptibility to catastrophe combine (Lacy, 1993) , an 'extinction vortex' (Gilpin and Soulé, 1986) may arise, whereby population numbers decline from generation to generation, partly because in any given generation population sizes depend partly on those in that immediately preceding it. With population extinction, resident genetic diversity is lost; as inbreeding depression due to depleted genetic variation is one consequence of small population size, reduced genetic diversity is at once both causal agent in, and effect of, population extinction.

Clearly, the impact of forest fragmentation on genetic diversity will partly determine the contribution that fragments can make to management and conservation of biodiversity, *i.e.* whether they can mitigate the many negative effects of deforestation, or whether they simply constitute a transient phase in forest destruction. Neither outcome is likely to apply to all species in all circumstances. Rather, given the diversity of plant and animal life, diversity of response would seem to be the most rational expectation. However, this also implies that certain characteristics and fragmentation scenarios are likely to be associated with the degree of resilience to fragmentation effects. The identification of such characteristics would seem to be the most promising means of arriving at meaningful generalizations on the effect of fragmentation and the potential of forest fragments to mitigate the effect of deforestation, although it should also be stressed that the drawing of such general conclusions should not be the only or, necessarily, the main objective of studies of forest fragmentation. In many cases, the response of individual endangered or valuable species may itself be of interest.

The present thesis is concerned with the genetic impact of forest fragmentation on tree species of the seasonally dry, Pacific watershed of Central America. Tropical dry forests and other forest ecosystems of this largely deforested (Janzen, 1986; Mooney *et al.*, 1995) zone now occur principally or wholly as fragments (Janzen, 1988). It follows that understanding of the consequences of fragmentation is particularly relevant to the rational management and conservation of the zone's biodiversity. Furthermore, its relatively high population concentration, including millions of rural and urban poor (FONTAGRO, 1997), as well as constituting one cause of its high degree of deforestation, is further justification for conservation of remaining biodiversity. Without such measures, the 'ecological, genetic, social, economic, scientific, educational, cultural, recreational and aesthetic values' mentioned above will cease to be available to millions of inhabitants of the region.

In Chapter Two, the context of the research is clarified by reviewing both the expected genetic consequences of forest fragmentation and results of studies to date. In Chapter Three, temporal and spatial patterns of forest removal in the study zone, together with their expected genetic consequences, are described. Subsequently, in Chapters Five and Seven, research on the effect of forest fragmentation on genetic and reproduction of two tree species of the zone, *Anacardium excelsum* (Anacardiaceae) and *Plumeria rubra* (Apocynaceae) is described; the preceding Chapters Four and Six describe the inheritance, linkage relationships and selective neutrality of the allozyme markers used in the studies. In the final chapter, the wider genetic implications of forest fragmentation in lowland Guanacaste are considered, taking into account both the results detailed in Chapters Three, Five and Seven, and the characteristics of tree species of the study zone.

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Chapter Two

THE GENETIC EFFECTS OF FOREST FRAGMENTATION: AN OVERVIEW OF EXPECTATIONS AND FINDINGS

INTRODUCTION

High rates of deforestation prevail in many tropical countries (FAO, 2001). However, in many cases, one or more tracts within formerly continuous tree cover remain forested (Riitters *et al.*, 2000; Schelhas, 1996). Deforestation converts such tracts to fragments set in an unforested matrix. If viable, communities and populations in such fragments have the potential to substantially mitigate the impact of deforestation on biodiversity.

The viability of any population is partly dependent on the maintenance of genetic variation, as the magnitude of the latter may be correlated with both individual and population fitness (Crnokrak and Roff, 1999; Frankham and Ralls, 1998). In fragmented populations, genetic variation and, therefore, viability is threatened due to pressures on key parameters (e.g. population sizes, genetic diversity) and processes (*e.g.* pollination, mating, gene flow). In the first part of the present review, these pressures and the mechanisms by which they are expected to affect genetic diversity are described. Subsequently, the results of field studies of effects of fragmentation on genetic diversity are reviewed in the context of these expectations.

REDUCTION IN POPULATION SIZE

For many species, forest fragmentation is likely to lead to reduction in population sizes. There are at least three ways in which this can occur. First, population members may be eliminated by the forest removal that gives rise to fragmentation. When migration, *i.e.* pollen and seed movement, is maintained between fragments, such population reduction is defined simply by the proportion of the population formerly located in the now deforested matrix. By contrast, total interruption of gene flow leads to a second, more severe, type of population reduction, in which a large, continuous population is reduced to a set of small populations, these corresponding to the fragments. Third, in both cases, initial population reduction may quickly be reinforced by pressures resulting from the profound environmental changes often wrought by fragmentation. Essentially, these occur because, when a tract in continuous forest becomes a fragment, there is necessarily a change in the adjoining habitat(s): it is logical to expect that the newly conjoined habitats will cause changes in each other, particularly if they differ markedly. There is growing evidence on the importance of the effects of unforested matrices on forested fragments, to such a degree that Gascon and Lovejoy (1998) remark that 'recent knowledge about…how the tropical rainforest ecosystem is affected by landscape fragmentation suggests that much of the ecological degradation can be accounted for by the influence of edge effects and the surrounding matrix'. Laurance *et al.* (1997) describe how such effects are strong enough to lead to biomass reductions of up to 36 per cent within 100m of fragment edges, in spite of increased biomass of lianas. Although such effects may increase population size of 'gap species' (e.g. Sizer and Tanner, 1999; Cunningham, 2000), they imply degradation of habitat of later successional species. For example, Jules (1998) found 'almost no recruitment' to *Trillium* populations within 65m of fragment edges in the Siskeyou Mountains, Oregon (interior recruitment was higher). Similarly, Mesquita *et al.* (1999) found a highly significant relationship between tree mortality and distance to edge in Amazonian forest fragments, whilst Gigord *et al.* (1999) found reduced numbers of juveniles in small populations of the Madagascan endemic *Dombeya acutangula* ssp. *acutangula*, due to increased competition from invasives.

Expected genetic consequences of reductions in population size

In finite populations, formation of successive generations tends to involve an element of sampling, because the number of gametes and/or zygotes is generally much greater than the adult population to which these give rise. Although viability selection may influence the genetic composition of the survivors, in general random factors will determine survival before natural selection is able to act. As with any sample, there is no guarantee that it will accurately reflect the genetic constitution of the population (in statistical and biological senses) from which it has been drawn. Consequently, unless gametes are genetically invariable, there will be random fluctuations in allele and genotype frequencies from generation to generation, *i.e.* random genetic drift. Eventually, random genetic drift leads to fixation and loss of alleles; fixation probability of each allele is equal to its initial frequency (Hartl and Clark, 1989), whilst expected time to loss depends both on this parameter and the effective population size (N_e), *i.e.* the number of individuals in a hypothetical 'ideal' population with the same magnitude of random drift as the actual population (Hartl and Clark, 1989, Savolainen and Kuittinen, 2000). For example, for p=0.5, expected time to fixation (or loss) is 2.8N_e generations, whilst for p=0.1 expected time to loss is about 1.3N_e generations (Yeh, 2000). In the interim, allele frequencies tend to become more extreme. As a consequence, heterozygosity is expected to decrease in each generation by a proportion of 1/2N_e, such that in generation t, expected value of gene diversity is:

$$H_{e(t)} = \left(1 - \frac{1}{2N_e}\right)^t H_{e(0)},$$

where $H_{e(0)}$ is initial gene diversity (Hartl and Clark, 1989). Additive genetic variance is expected to decline at the same rate (Lande and Barrowclough, 1987, Savolainen and Kuittinen, 2000). Otherwise, however, the course of drift in an individual population is impossible to predict. For example, if the least common allele increases in frequency, expected heterozygosity will show a transient increase, although eventually a chance sequence of events is expected to lead to fixation and therefore loss of all heterozygosity.

In practice, and for two reasons, complete fixation is unlikely to occur. Firstly, mutation may reintroduce lost alleles, although, given the low frequency of mutation events, for small population sizes equilibrium values of heterozygosity in closed finite populations will still be close to zero. Secondly, analogously, and more potently, migration can also introduce alleles. This second factor is of greater importance: both generally, given that migration rates are generally several orders of magnitude higher than mutation rates (Hart and Clark, 1989), and in the present context, because the genetic effect of forest fragmentation has been seen as depending on the interaction of genetic drift (reducing variation) and migration (introducing variation). This interaction is further discussed below ('Fragmentation and gene flow').

Post-fragmentation decline in population numbers further increases susceptibility to drift. However, such effects do not derive only from direct reductions of population size, because the magnitude of genetic drift can be predicted as a simple function of census population size only when the population characteristics meet the assumptions of the Fisher-Wright drift model (Caballero, 1994). Usually, this is not the case (Hartl and Clark, 1989), and consequently N_e tends strongly to be smaller than N (Frankham, 1995). Several factors are responsible for this tendency, including unbalanced sex ratios, non-Poisson distribution of fecundity and population size fluctuations (Falconer, 1989; Futuyma, 1986; Hartl and Clark, 1989; Lande and Barrowclough, 1987; Nunney, 1993; Wright, 1938; Yeh, 2000). Of these, the most important is generally considered to be variance in fecundity (Nunney, 1993; Falconer, 1989). There are good reasons to expect fragmentation to affect the ratio $\frac{N_e}{N}$, principally by introducing more highly variable environmental conditions, typified by the edge-interior distinction: just as fragmentation may increase or decrease fertility, so it can be expected to increase variation in fertility, because the environmental changes which lead to the increases or decreases may not be uniform within fragments. Aldrich and Hamrick's (1998) work illustrate this effect. They found that reproduction of *Symphonia globulifera* (Clusiaceae) in a 38.5ha circular plot was dominated by a numerically small group of remnant pastureland trees, which experienced a post-fragmentation increase in fecundity, leading to a 'secondary constriction' of the fragmentation bottleneck. Similarly, in the New Zealand mistletoe *Peraxilla tetrapetala* (Loranthaceae) pollination and seed set were more than fourfold higher in isolated than continuous-forest individuals (Kelly *et al.*, 2000).

When fragmentation leads to immediate reductions in population size, 'one-time drift' or 'founder effects' (Yeh, 2000) may occur, because remaining trees constitute a sample of the original population. The expected average number of alleles remaining after a founder event is:

$$E = A - \sum_j (1 - p_j)^{2N} ,$$

where A = the number of alleles (allelic richness) before population reduction, p_j =frequency of the jth allele, N=population size after reduction (Meffe and Carroll, 1994). That is, it depends on the initial number and frequency of alleles. Under the infinite alleles model of mutation, for likely values of θ ($\theta=4N_e\mu$, where μ=mutation rate), populations tend to have no more than 2-4 alleles at frequencies > 0.05 (Marshall and Brown, 1975). Frankel and Soulé (1981) provide examples of the effects of one-time drift on initial allele frequencies consistent with these theoretical expectations. For example, for p=0.94 and q=r=s=0.02, two of four alleles are lost on reduction of population size to 10 individuals, whilst reduction to 50 individuals leads to 10% loss of allelic richness.). S-alleles, *i.e.* those controlling self-incompatibility mechanisms, may be particularly susceptible to loss through founder effects as, due to frequency-dependent selection, allelic richness tends to be high, *i.e.* allele frequencies tend to be low. A decrease in allelic richness at incompatibility loci will tend to reduce fertility, as well as exerting selective pressure in favour of occasional self-compatible individuals.

By contrast, gene diversity is expected to be relatively little affected by bottlenecks, because rare alleles contribute little to total heterozygosity; as the expected reduction is $\frac{1}{2N}$, 95 percent of gene diversity is conserved in a bottleneck of ten individuals (Frankel and Soulé, 1981). The latter authors comment 'unless the number of founders is of the order of two pairs or fewer, the bottleneck, *per se*, is not the villain...most of the loss that ensues is attributable to events following the bottleneck'.

The above formulation implies that the fragmentation-mediated 'sampling' process is analogous to simple, unbiased random sampling. In practice, this is unlikely to be the case. Firstly, fragmentation is patently not analogous to simple random sampling, because it 'samples' spatially clustered groups of trees, not individuals distributed randomly in space. As limited seed dispersal in forest trees frequently leads to small-scale spatial genetic structure (Hamrick *et al.*, 1993; Loiselle *et al.*, 1995; Hamilton, 1999), fragmentation may lead to loss of more genetic variation than if a similar number of trees was randomly removed from the population. Secondly, if, as is frequently the case, fragmentation is non-random with respect to environmental conditions (e.g. soil type) (Laurance, 1999; Chapter Three), local adaptation to specific sites (Gilbert *et al.*, 1996; Linhart and Grant, 1996) could generate correlations between spatial genetic structures and deforestation criteria (*i.e.* those factors, such as soil conditions, which determine whether a given forest tract is removed), leading to loss of particular non-neutral alleles or allelic complexes (Nason *et al.*, 1997).

FRAGMENTATION AND POLLINATION

Fragmentation leads to changes in abundance of many insects, including pollinators (Didham *et al.*, 1996). Particularly in tropical forests, where more than 90 per cent of trees are thought to be animal-pollinated (Bawa *et al.*, 1985), effects of fragmentation on pollination are therefore to be expected. At least five specific mechanisms may be identified.

First, small fragments may be too small to support resident pollinator populations: possible causal factors include low numbers of particular plant species (specialist pollinators) or absence of high density resources (many generalists), lack of suitable nesting habitat and larval host plants, pesticide contamination from agricultural matrices, or invasion of competitors or predators (Murcia, 1996; Nason and Hamrick, 1997; Rathke and Jules, 1993; Roubik, 1989).

Second, non-resident pollinators are less likely to visit small, isolated populations, as they offer lower resource levels, require more energy to reach, and may not even be detected. For example, Mustajärvi *et al.* (2001) found that bumble-bees preferred larger artificially established fragments of the outbreeding, self-compatible *Lychnis viscaria.* Groom (1998) reported that small patches of *Clarkia concinna* received fewer pollinator visits when certain isolation thresholds were reached, leading to reproductive failure due to lack of pollination. Jennersten (1998) found that *Dianthis deltoides* flowers in a fragmented area received fewer pollinator visits and set less seed than in a 'mainland' (unfragmented) area; hand pollination increased seed set, an indication of pollinator limitation (Vaughton and Ramsey, 1995). Similarly, Ågren (1996) found positive relationships between population size and seed production per flower and plant in the herb *Lythrum salicaria*, caused by pollinator limitation.

Third, forest destruction may lead to increased mean pollinator movements, as pollinators are forced to forage further afield. Fourth, fragmentation-induced changes in plant density (which may result from reductions of population size within a finite area) may cause changes in pollinator behaviour. For example, in both the wetland perennial *Mimulus ringens* (Karron *et al*, 1995) and the tropical tree *Shorea siamensis* (Ghazoul *et al.*, 1998), the proportion of pollinator flights between (as opposed to within) plants was positively correlated with plant density. Finally, similar effects could also be caused through changes in pollinator species assemblages. For example, both Aizen and Feinsinger (1994a) and Dick (2001) reported fragmentation-caused increases in importance of feral honey-bees. Such changes could be beneficial in some cases. Dick's report, for example, describes the positive role of *Apis* as long-distance pollinators able to effect pollination in small and isolated fragments. Aizen and Feinsinger (1994a), by contrast, found that *Apis* bees tended to move between trees more rarely than native bees, whilst Gross and Mackay (1998) found that honey-bees reduced fitness in the Australian pioneer shrub *Melastoma affine* by disturbing the more effective native pollinators.

It should not be assumed that fragmentation will always be associated with changes in pollinator abundance and behaviour. Rather, the incidence of such effects is likely to depend on particular circumstances, e.g. fragment size, isolation and pollinator mobility. For example, for 14 species present in Andean cloud forest remnants, Murcia (1996) reported no differences in pollination between small and medium-sized fragments. She suggested that it is likely that pollination will be affected only when fragments reach extremely small size. Similarly, Aizen and Feinsinger's (1994b) study of effects of fragmentation on pollination of 10 species of Argentinian dry forest revealed that pollination decline was greatest in those species which receive few visits even in continuous forest.

Patterns of pollen transfer between trees affect both gene flow and mating systems. In the case of the latter, in self-compatible species, increases in within-plant movements would be expected to reduce outcrossing rates, due to increased geitonogamous selfing. Empirical results are consistent with this expectation. For example, Murawski and Hamrick (1992) and Murawski *et al.* (1990) established that outcrossing rate in *Cavanillesia platanifolia* is density-dependent: isolated trees had high selfing, whereas trees in groups were predominantly outcrossed. Similarly, Prober and Brown (1994) found negative relationships between tree density and values of Wright's fixation index in fragments of *Eucalyptus albens.* Aldrich and Hamrick (1998) found that pasture trees of *Symphonia glabra* showed higher selfing rates than neighbouring conspecifics in continuous forests, and attributed this to changes in pollinator foraging patterns. Population density effects have also been implicated in interpopulation variation in outcrossing rates in *Pterocarpus macrocarpa* in Thailand (Liengsiri *et al.*, 1998).

Increased selfing leads to decreases in effective population size (Yeh, 2000) and increased homozygosity, with the attendant risk of inbreeding depression.

The possible impact on gene flow of changes in pollinator abundance and behaviour is discussed below, following a brief outline of migration theory.

FRAGMENTATION AND GENE FLOW

The magnitude and patterns of gene flow are central to the genetic effects of forest fragmentation, because gene flow may act to counter random genetic drift. This is not simply because immigration can reintroduce alleles lost by drift. As outlined below, if migration is sufficiently prevalent, 'separate' subpopulations may in effect behave as one larger population, thus reducing the importance of genetic stochasticity.

Theoretical formulation and investigation of the interaction between migration and drift has relied principally on three models, *i.e.* Wright's Isolation by Distance (IBD) model, Kimura and Weiss's Stepping Stone model, and Wright's Island model (Hamrick, 1987; Lande and Barrowclough, 1987). IBD applies to continuously distributed populations, whereas the latter two apply to spatially discrete subpopulations, such as those found in forest fragments. The Island and Stepping Stone models differ principally in the assumed mode of gene exchange between subpopulations. The former assumes that migration occurs at the same rate between all subpopulations. As such, it represents 'the extreme in long distance gene flow' (Hamrick, 1987). The Stepping Stone model, by contrast, assumes that gene flow occurs predominantly between adjacent populations; as pointed out by Wright (1969) himself, it is therefore more realistic. However, in the case of a two-dimensional spatial configuration of subpopulations, theoretical investigation of both models leads to a similar conclusion, *i.e.* that 'remarkably' little gene flow between subpopulations is required to prevent genetic drift (Hartl and Clark, 1989). Under the Island Model, numbers of immigrants as low as $mN_e>2$ severely limits genetic divergence (Hartl, 2000), whereas, in the case of the Stepping Stone Model, Crow and Aoki's (1984) work demonstrated that only one to two migrants per generation are required to maintain overall panmixia. The difference between the two models is more significant when spatial configuration approximates the one-dimensional case, as, for example, in ridge-tops or fragmented riparian habitat. In this case, the more realistic Stepping Stone model produces markedly different results to the spatially 'blind' Island Model: considerably more gene flow per generation is required to maintain overall panmixia, because the correlation between neighbouring demes is higher (Hartl and Clark, 1989; Wright, 1969). For example, in a Stepping Stone model with migration rates of 0.1 and (2)(10^{-5}) respectively for adjacent and long-distance gene flow, 'considerable' local differentiation will occur if $N_e<100$ (Kimura and Weiss, 1964).

The main findings of the Island and Stepping Stone models in two-dimensional spatial configurations have received practical expression in the 'one-migrant-per-generation' (OMPG) rule, as applied 'in the USA [in] nearly every recovery plan that considers genetic issues and insularization' (Mills and Allendorf, 1996). The OMPG rule seeks to attain a balance between subpopulation and total diversity, *i.e.* avoiding both loss of alleles within subpopulations and the uniformity associated implied by complete panmixia. An equilibrium fixation index value of F_{st}=0.2 is considered to achieve this balance, corresponding to $mN_e=1$.

Many studies of forest fragmentation have tended to assume tacitly or implicitly that spatial isolation by fragmentation would also lead to reproductive isolation, or at least to some reduction in numbers of immigrants. There appear to be good reasons for such an assumption. Firstly, in many species, fragmentation necessarily involves the removal of one source of immigrant pollen, *i.e.* those trees formerly located in the deforested matrix. Secondly, as outlined above, fragmentation may lead to reductions in pollinator abundance, due both to lower populations of resident pollinators and reduced visitation. In themselves, both factors will tend to reduce the amount of external pollen or seed entering a given fragment. Unless fragmentation leads also to at least equivalent reductions in pollination events involving 'home' pollen, this will lead to decreases in migration rate and, consequently, reductions in mN, the gene flow parameter. However, it is worth stressing that, as suggested above, such reductions in 'home' pollination constitute one expected consequence of fragmentation, and therefore it is questionable whether an assumption of increased reproductive isolation is justified. It should also be mentioned that pollinator populations themselves may show relatively short-term evolutionary responses to fragmentation, e.g. adaptations for increased mobility (Van Dyck and Matthysen, 1999).

Recent studies of pollen dispersal in fragmented landscapes, which have demonstrated that long-range pollen flow is common in tropical trees, lend force to this contention. For example, Apsit *et al.* (2001) reported mean pollen flow over three years of >70 per cent to a *Enterolobium cyclocarpum* population, in spite of the fragmented surrounding landscape, and commented that 'pollen dispersal distances of 1500m may be relatively common in *E. cyclocarpum*'. Dick (2001) reported *Apis*-mediated pollen flow of up to 3.2km to *Dinizia excelsa* (Fabaceae) fragments. White and Boshier (2000) reported that 70 per cent of pollen flow to an isolated *Swietenia humilis* tree came from a stand located 4.5km away, whilst Nason and Hamrick (1997) reported that, in small island populations of *Spondias mombin*, 90-100 per cent of progeny produced was the product of pollen flow from 80-1000m away.

Although such extensive post-fragmentation gene flow does not necessarily imply either reduced, increased or static levels of gene flow relative to pre-fragmentation conditions, it does imply that, in such fragments, genetic drift is less likely to threaten population viability. At the same time, it would be unwarranted to conclude definitively that migration will always remove the possible threat of genetic drift in fragmented populations. Firstly, migration rates are not necessarily independent of population size. At the extreme, for population size N=1 of any self-incompatible species, migration rate cannot be less (or more) than 50 per cent, whilst for any small fragment population, extrafragment trees may greatly outnumber population members, leading to the possibility of high expected gene flow, when this is not precluded by pollinator foraging capability. Such relations may not apply in larger populations.

Secondly, migration numbers well in excess of 'OMPG' may be insufficient to halt centrifugal tendencies. Mills and Allendorf (1996), in their critique of unquestioning application of the OMPG rule, suggest that failure in the assumptions of the Island model (Whitlock and McCauley, 1999) imply that a more suitable general rule of thumb would be 'a minimum of 1 and a maximum of 10 migrants per generation'. This, or even higher numbers, would apply particularly in the case of gene flow in spatial configurations tending more to the one-dimensional than two-dimensional case.

Thirdly, the expected mitigatory effect of gene flow applies principally to the equilibrium situation. Insofar as prefragmentation populations are in equilibrium, a sudden reduction in genetic diversity, but with unchanged gene flow, constitutes a disturbance of equilibrium, *i.e.* genetic variation will be less than expected for prevailing gene flow. Over time, gene flow is expected to restore genetic variation until the point of equilibrium is reached. However, in the interim, which can last many generations (Whitlock and McCauley, 1999) populations will still be subject to reduced genetic variation, with potentially serious results (for example, loss of S-alleles).

Evidently, the genetic effects of forest fragmentation are not easily predictable based on theory, even when reinforced by empirical observations of processes with genetic implications. Evaluation of the gravity of the genetic threat posed by fragmentation can only be satisfactorily evaluated by taking into account empirical studies.

GENETIC VARIATION IN FRAGMENTED PLANT POPULATIONS

Two broad approaches of detecting effects of fragmentation on genetic variation have been used. Firstly, comparison of fragmented populations with unfragmented populations, *i.e.* either comparable populations in unfragmented landscapes, or prefragmentation cohorts of the same populations. Secondly, relationships between measures of genetic variation and indices of intensity of fragmentation (*i.e.* typically, population size or, occasionally, isolation) have been studied. Studies of these sorts have produced diverse results. However, in several cases, important genetic effects have been detected, whilst, when this has not been the case, the lack of effect is generally explicable in terms of probable values of the factors outlined above.

Fragmentation has been associated with declines in allelic richness in a number of cases. For example, in 17 fragmented populations (N from 1 to 430) of the perennial *Swainsona recta*, Buza *et al.* (2000) reported significant effect of log population size (R^2=0.70, p<0.001), due to absence of rare alleles from small populations. Similar relationships were reported by Van Treuren *et al.* (1991) (respectively 14 and 12 populations of the bee-pollinated perennials *Salvia pratensis* and *Scabiosa columbaria*, N=5-1500, 14-100,000, r=0.57, 0.65) and Prober and Brown (1994) (25 fragments of *Eucalyptus albens*, N from 14 to >10000, R^2=0.39, p=0.007). Raijmann *et al.* (1994) (25 populations of *Gentiana pneumonanthe*, N from 6-100,000) reported A_e rather than A. They found positive correlations between log population size and both this parameter and

P, the proportion of polymorphic loci, although the relationships with A_e was only marginally significant (A_e: r=0.33, p=0.105; P: r=.49, p=.013). The case of the daisy *Rutidosis leptorrhynchoides* (Young *et al.*, 2000) is of special interest. In this case, not only was overall allelic diversity in 17 fragment populations (N=5-95,000) strongly positively related to log population size, but fragmentation also led to loss of incompatibility alleles. The consequences of this extend beyond simple loss of fertility. In the authors' words: 'firstly…reduced S-allele richness lowers the effective population size, further exposing populations to genetic drift. Secondly…low mate availability may reduce population-level seed-set while increasing interplant variance in fecundity…Thirdly, severe mate limitation will also favour self-compatible plants that occur at low frequencies'. Effects of fragmentation on diversity of S-alleles has also been implicated in local extinctions of the Great Lakes lakeside daisy, *Hymenoxys acaulis* var. *glabra* (Demauro, 1993).

In all the above instances of strong relationships between population size and allelic richness, the former parameter ranged from very small (*i.e.* <10) to several orders of magnitude greater. In studies in which no relationship has been detected, either the range of population sizes has tended to be less or, perhaps more importantly, very small populations were not included (e.g. *Festuca ovina* (N of 25-980, *Lychnis viscaria* (10-680), *Arabis thaliana* (N of 2 to >100) (all Berge *et al.*, 1998), *Acacia anomola* (N=3-50) (Coates, 1988), *Arnica montana* (N=20-1500) (Kahmen and Poschlod, 2000), *Microseris lanceolata* (N=87-140,000) (Prober *et al.*, 1998).

In many cases, loss of allelic richness has been attributed to founder effects. Although gene flow between fragments might restore lost alleles relatively quickly (conceivably, in the first reproductive events after fragmentation), loss of non-neutral variation is also likely to affect fitness relatively quickly. Furthermore, alleles can only be restored if present in the post-fragmentation population as a whole. Young *et al.*'s (1993) work with *Acer saccharum* illustrates this point. Although fragmentation appeared to increase gene flow between fragments and, consequently, individual fragments tended to be more similar than the controls (tracts in continuous forest), the total number of alleles over all fragments was less than in the tracts of continuous forest, apparently because founder effects led to the loss from the fragmented area of the alleles in question.

As outlined above, gene diversity is not expected to be as sensitive to founder effects. Nevertheless, decline in values of this parameter have been reported. In Prober and Brown's (1994) study of *E. albens* (1994), log population size was more strongly related with gene diversity than with allelic richness (R^2=0.49, p=0.001). A relatively high correlation were also reported by Raijmann *et al.* (1994) for 25 populations of *Gentiana pneumonanthe* (r=0.34, p=0.09). However, in a number of other studies of relatively-recently fragmented populations, there were no clear relationships between gene diversity and log population size (Berge *et al.*, 1998; Foré *et al.*, 1992; Young *et al.*, 1993, 1999, 2000; Buza *et al.*, 2000; Van Treuren *et al.*, 1991; Coates, 1998; Kahmen and Poschold, 2000). These results contrast with those from long-isolated, naturally-fragmented species. For example, Sampson *et al.* (1998) found low genetic diversity and high population differentiation (G_{st}=0.24) in isolated populations of the granite-outcrop species *Eucalyptus crucis*, whilst in populations of the dioecious conifer *Halocarpus bidwilii*, estimated to have been naturally fragmented for about 8000 years (*i.e.* ≈100 generations), gene diversity was significantly related to log population size (p=0.004, correlation coefficients not reported) (Billington (1991).

Both *E. albens* and *G. pneumonanthe* are self-fertile, and both of the mentioned studies reported evidence of increased selfing in fragmented populations. At least in the case of *G. pneumonanthe*, there is also evidence of higher selfing in small populations (see below). Such effects would also enhance relationships between population size and H_e (*i.e.* beyond the expectation for drift alone), as observed by Young *et al.* (1996) in connection with these two studies. This suggestion finds some support in the other mentioned studies. *F. ovina* (Berge *et al.*, 1988), *R. leptorrhynchoides* (Young *et al.*, 1999) and *A. montana* (Kahmen and Poschlod, 2000) are self-incompatible, whilst *A. thaliensis* (Berge *et al.*, 1998) is predominantly selfing. In these cases, there is no reason to expect correlation of selfing rate with population size, and therefore no reason why inbreeding should contribute to the gene diversity - population size relationship. Furthermore, in the cases of (possibly) self-compatible or partially self-compatible species such as *Acer saccharum* (Foré *et al.*, 1992), *Lychnis viscaria* (Berge *et al.*, 1998) and *Swainsona recta* (Buza *et al.*, 2000), such relationships are likely to be mediated by pollinator behaviour, and, rather than being axiomatic, are conditional on size and distribution of fragments.

A number of studies has examined the relationships between genetic variation and fragment isolation. For example, in *A. saccharum* patches (mean isolation of 58.9m), Foré *et al.* (1992) reported a significant positive relationship between number of canopy trees and observed heterozygosity of juveniles in highly-isolated patches (*i.e.*≥65m), suggesting that, in more isolated patches, gene flow may be insufficient to counteract factors responsible for higher observed homozygosity. Juveniles in highly isolated patches also had a significantly greater proportion of monomorphic loci. Prober and Brown (1994) found that the combination of log population size and distance to the nearest large stand of *E. albens* explained 48 per cent of variation in allelic diversity, suggesting the presence of 'isolation thresholds' beyond which fragmentation effects might occur. Similarly, Hall *et al.* (1996) in their report on genetic diversity and differentiation among nine populations of *Pithecellobium elegans* in Costa Rica found that genetic variation in the small fragment closest to a large population was greater than that in the small fragments further away, suggesting the presence of 'important genetic links', and implying that in the other fragments the degree of isolation has eroded genetic variation, or at least not permitted its postbottleneck restoration. Dayanandan *et al.* (1999) found that genetic distance between adult and seedling cohorts in fragment populations of *Carapa guianensis* (Meliaceae) in Costa Rica was greatest in the most isolated population, which was also the only one in which allelic diversity was higher in the adult cohort. In other studies, relationships between isolation and diversity have been absent (Buza *et al.*, 2000; Young *et al.*, 1999).

Finally, several studies have reported inbreeding depression in fragmented populations. In *Swainsona recta*, Buza *et al.* (2000) found negative correlations between population size and fixation indices, and negative correlations between the latter and germination percentages. Fischer and Matthies (1998) found that remnant population size in *Gentianella germanica* was correlated with plant survival in a common garden experiment (Spearman r=0.50, p<0.05). Gigord *et al.* (1998) documented inbreeding depression on selfing in the Madagascan endemic tree *Dombeya acutangula* ssp. *acutangula*: outcrosses produced more fruits per flower, more seeds per fruit and lower proportions of flattened, lighter seeds. In a comparison of seven populations, population size was strongly positively correlated with one of these variables (mean number of seeds per fruit). Although between-population variation in proportion of flattened seeds was not significant, mean proportion in the three smallest populations (N≤10, mean 15.3% flattened) were notably higher than in the largest population (*N*=81, 5.4% flattened). In seven small populations of the self-compatible *Gentiana pneumonanthe*, Raijmann *et al.* (1994) reported a significant correlation between selfing-rate and log population size, apparently caused by decreased visitation of the relatively non-mobile bumblebee pollinator. As heterozygosity is known to affect fitness in this species (Oostermeijer *et al.*, 1994), this result implies inbreeding depression in small populations of this species.

CONCLUSIONS

Clearly, fragmentation effects on population genetics of forest trees and other plants are complex and difficult to predict. In particular, theoretical considerations are perhaps more useful in understanding and rationalizing, *i.e. a posteriori*, empirical results, rather than predicting them. However, it is worth emphasizing that both expectations and findings suggest that fragmentation can exert a rather rapid effect on genetics of fragmented populations, both through effects on pollinators and founder effects. Although the theoretical formulation of random genetic drift in subdivided populations appears to correspond closely to fragmentation, it does not and is not intended to describe all consequences of population subdivision. Consequently, genetic response to fragmentation will not necessarily occur only in long-term, and neither will it necessarily be easily mitigated by gene flow.

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Chapter Three

HISTORICAL AND SPATIAL PATTERNS OF DEFORESTATION AND FRAGMENTATION IN GUANACASTE PROVINCE, COSTA RICA: CHARACTERIZATION AND GENETIC IMPLICATIONS

INTRODUCTION

The seasonally dry tropics have been heavily deforested (Mooney *et al.*, 1995). In Mesoamerica, this trend is particularly marked, due largely to high human population concentration (Murphy and Lugo, 1995). According to Janzen (1986), only around 2 per cent of the original forest cover remains. However, even in such heavily disturbed zones, deforestation of a given area is rarely complete (Schelhas, 1996). Rather, one or more tracts within the original continuous forest remain. If viable, communities and populations in such fragments have the potential to mitigate the impact of deforestation on biodiversity. However, such viability is not assured, because small populations are subject to a number of pressures which are absent or of less importance in large populations (Soulé, 1987). These include several factors which, if present, have the potential to reduce genetic diversity in fragmented populations (see Chapter Two). This is of concern because genetic erosion may lead to decline in fitness and evolutionary potential (Brook *et al.*, 2002; Frankham and Ralls, 1998; Young *et al.*, 1996).

The genetic impact of fragmentation depends in part on its spatial and temporal patterns. Both partially define the fragmentation state and process, and thereby determine the intensity of fragmentation. Because the latter is a spatial phenomenon, this is most clearly seen in the case of spatial patterns. However, temporal patterns are also of great interest, as the effect of evolutionary processes depends not only on their intensity but also on the number of generations for which they act.

In the present document, historical and spatial patterns of forest fragmentation in a zone of seasonally dry, northwestern Costa Rica are characterized. After a description of the study area, forest history since the Spanish conquest is traced, based on published sources, maps and aerial photos, culminating in a quantitative analysis of forest change and state since the 1940s. Finally, the biological implications of the findings are discussed, with particular emphasis on genetic aspects.

THE STUDY ZONE

The study zone, an approximately 350km^2 rectangular section of the Pacific slope of Costa Rica (Figures 3-1, 3-2), lies between latitudes 10°35’ and 10°17’*N* and longitudes 85°05’ and 85°12’*W*. It rises from sea level at its southern limit, some 12km north of the Gulf of Nicoya, to 250m a.s.l. just north of the village of Palmira, approaching the foothills of the Miravalles and Tenorio volcanoes. Around 95 per cent of the mean annual rainfall of 1693.4mm (s =459.4) falls between May and November (San Luis, Cañas meteorological station, 1921-1978, MIRENEM, 1988). Altitude varies from 0-200m a.s.l; mean annual temperature at 95m a.s.l. is 27.5°C (Jiménez *et al.*, 1987). The dry season is characterized by strong (up to 90km hr^{-1}) northerly winds (Coen, 1983) and temperatures up to 37°C. Soils in the study zone fall into two broad categories: south and immediately north of the Interamerican Highway are mollisols, alfisols, vertisols and alluvial inceptisols of generally medium to high agricultural potential, although with some drainage problems. An escarpment three to four kilometres north of the Highway marks a transition to shallower inceptisols, some derived from deep volcanic ash deposits (Oficina de Planificación Sectorial Agropecuaria, 1987). Within both areas, topography is generally flat to undulating. Two main land-uses predominate in the study zone, reflecting the soil differences mentioned above: the southern zone is dominated by latifundist industrial agriculture (flooded rice and sugar-cane) whilst the northern zone is dominated by beef-cattle ranching, with mixed land tenure. The rivers Cañas, Corobicí, Tenorio, Tenorito and their numerous tributaries flow in a predominantly southwest direction across the study zone, draining into the River Bebedero and thence to the Gulf of Nicoya. They form deep ravines and canyons in the tuff overlaying parts of the northern sector of the study zone. Forest is the natural vegetation of the entire study zone, with the possible exception of some parts of the poorly drained vertisols in the southern sector. Naturally occurring species include a number of high commercial value, e.g. mahogany (*Swietenia macrophylla*), Spanish cedar (*Cedrela odorata*), rosewood (*Dalbergia retusa*) (Jiménez et al., 1987; see also Appendix 3). The city of

Cañas, population 18,798 (INEC, 2002), is the only relatively large centre of population in the study zone. In addition, there are two larger villages: Bebedero (2123 inhabitants) and Palmira (916) (INEC, 2002). The former, situated at the confluence of the Corobicí and Tenorio rivers (Figure 3-2), is a long-established river port and has been settled since at least 1687 (IGN, 1972).

The study zone was selected primarily for studies of genetic impacts of fragmentation on two native species (see Chapters Five and Seven). Whilst no one single site can be considered representative of all of lowland, seasonally dry Costa Rica, land-use and topography reflect common features of the zone, e.g. industrial agriculture on high quality soils, prominence of cattle ranching, and the canyon rivers of the northern sector (*c.f.* Vargas Ulate, 2001).

The zone is known to have been inhabited at the time of the Spanish conquest in the early 16th century; Ibarra (1990) places it within the Zapandí and Corobicí tribal areas. Consequently, it is unlikely that it was undisturbed during pre-Columbian times. In particular, clearance of lands located close to permanent watercourses, e.g. on the fertile mollisols close to the River Tenorio (Figure 3-2), seems likely. According to Meléndez (1955), such locations were preferred sites for indigenous habitation; indeed, the same author mentions the discovery of petroglyphs in the Paso Hondo farm, which is located in this area. In spite of this, there is no reason to believe that human activity in the pre-Columbian period led to extensive deforestation or forest fragmentation. There appear to be no records of major population centres within the study zone, whilst descriptions of the subsequent colonial period correspond to those of a very largely forested (pre-Columbian) landscape in the process of conversion to the largely deforested one of today.

This deforestation process is traced here based on two principal sources of evidence: general accounts of the agrarian history of Guanacaste province, and specific references to the study zone. Although the latter are less complete than the former, joint consideration of both sources of evidence is informative. Reconstruction is also facilitated because many of the general references apply specifically to the area known historically as the Bagaces Valley, of which the study zone forms a part.

GENERAL PATTERNS OF DEFORESTATION IN LOWLAND GUANACASTE

Deforestation in lowland Guanacaste can be described in three main phases, all associated with particular historical-agricultural and land-use developments, as described below.

Phase One: feral cattle ranching (1560-to late 19th century)

Livestock (*i.e.* equine and bovine) was first introduced to Guanacaste in 1561 (Fournier, 1992). However, the result was not the birth of an intensive cattle-based agriculture, but rather the founding of what would become a semi-feral cattle population that would be 'managed' essentially as a renewable natural resource for more than three centuries. In large measure, this was because low human population densities (in 1688, the population of the Bagaces Valley, which includes the study zone, was 297) and large distances to markets precluded more intensive husbandry (Edelman, 1992). Cattle roamed in unfenced, wooded areas known as *sitios*, owners having rights to a certain number of cattle thought to be grazing a particular *sitio*, plus a proportion of the 'natural increase' (Edelman, 1992).

Phase Two: Timber exploitation, introduction of exotic pasture and cattle (1880-1930s)

Between 1880 and 1920, there was a marked expansion (approximately tenfold) in timber production from Guanacaste (Edelman, 1992). As well as leading directly to forest destruction, much of the proceeds from timber exploitation were invested in pasture improvement (Acuña y Molina, 1991), *i.e.* sowing of exotic varieties which permitted year-round grazing and up to 300 per cent increase in stocking rate. Improved pasture was sown preferentially on fertile sites close to rivers, and seed was also broadcast in the *sitios* (Edelman, 1992). In 1909 Guanacaste was legally declared a 'livestock zone', i.e. in which the onus was on agriculturists to 'fence out' cattle in order to protect their crops, rather than cattle-owners being responsible for 'fencing in' (Edelman, 1992). Both timber extraction and cattle expansion must have contributed to further forest destruction and attrition. By 1940, according to Becker (1943), the extractive timber phase was 'complete on all privately owned land with easy access to the main rivers and their tributaries'. However, it should be noted that selective logging of commercially valuable species such as mahogany (*Swietenia* spp) and Spanish cedar (*Cedrela odorata*), which tend to occur at relatively low densities, is not likely in itself to have led to outright forest destruction.

Phase Three: Deforestation in the 1950s and after

Between the 1950s and the 1970s, the area of pastureland in Guanacaste more than doubled as a result of expansion in the beef trade (Edelman, 1992); the latter author stresses that, in comparison with this phase, deforestation in the 1880-1930 period was relatively insignificant.

DEFORESTATION IN THE STUDY ZONE

Phases One and Two

There is no doubt that the wider changes outlined above were manifested also in the study zone. This is particularly well established for the southern sector of the study zone. Edelman (1992) reported that concessions for grazing *sitios* were made in the Bagaces Valley as early as the 1560s (that is, coinciding with the introduction of cattle to Guanacaste). Much of the southern part of the study zone is occupied by one large holding, the Taboga (formerly Higuerón) *hacienda.* Taboga, together with the contiguous Paso Hondo property (now mostly broken up by land reform), are amongst the oldest established properties in Guanacaste, founded no later than 1712 and 1787, respectively (Gudmundson, 1983). According to Meléndez (1955), in 1792 both were amongst the main farms of the region. Although, in the period to 1611, livestock production was mostly equine (mules) rather than bovine (Quirós, 1990), by the early 18th century (at latest) this had changed: the traveller John Cockburn recorded seeing 'great herds of cattle' on the east bank of the Tempisque (Meléndez, 1974), whilst other documents record similar observations as early as 1719 (Meléndez, 1974, citing Fernández Bonilla, 1884, p. 315). Edelman (1992) comments that 'in the colonial period [*i.e.* to 1821], the lowland plain along the eastern bank of the Tempisque, between the towns of Cañas and Liberia, was the principal area of cattle production within the Costa Rican jurisdiction'. A number of *sitios*, now mostly no longer forested or wooded, are marked on the 20th Century 1:25000 and 1:50000 topographic maps of the study zone, e.g. Sitios de Paso Hondo (IGCR, 1956a), Sitio Paraiso (IGCR, 1956b), Sitios de la Uvita (IGCR 1956c), Sitio Cascante (IGN 1973). They testify eloquently to the destruction of the zone's forest, as well as its previous use for extensive grazing.

Taken as a whole, the above information indicates that much of the southern part of the study zone was subject to grazing pressure from an early date. The effect of such pressure was recorded by the traveller von Seebach, who visited the area in 1864 (Meléndez, 1974). He refers specifically to the crossing of the Duquesa and Reventado rivers (Figure 3-2), and described the surrounding countryside as 'not very dense and … interspersed with small pastures' (Meléndez, 1974).

It is also clear that the zone was affected by the phase two changes. There was year-round grazing (i.e. based on exotic pasture grasses) in Hacienda Mojica (bordering the River Tenorio) by 1885 (Gudmundson, 1983), whilst the same author reports introduction of exotic pastures in the Paso Hondo farm in the 1930s. Edelman (1992) reports that between the 1920s and the 1930s Julio Sánchez Lepiz had 13,624 ha dedicated to (Nelore) cattle in the Taboga farm. With regard to the timber expansion, León (1952) wrote that Bebedero, effectively an enclave between the Mojica and Paso Hondo *haciendas*, was the most important river port in the timber trade. It would be odd if the timber exported did not include the product of the neighbouring properties themselves, a conjecture supported by Glander and Nisbett's (1996) comment that mahogany and other species were being logged at Paso Hondo during the 1920s. Independent of the causes - pasture conversion or logging - the major point is that there is evidence that large sections of the southern sector of the study zone were already deforested before the major cattle expansion of the 1950s. Céspedes Marín travelled the road from Bebedero to Cañas in 1923, and describes the wide, open plains ('planicie tan extenso') interspersed with occasional ('uno que otro') trees (Meléndez, 1974); given his route, this description is almost certainly of the Mojica, Paso Hondo and Taboga properties. More clear still, although recording a later date, is the evidence of the 1:25000 map series, published in the 1950s but based on aerial photos from 1945, which shows that by this date much of the area of these two properties was already deforested (IGCR 1955, 1956a, 1956b, 1956c, 1956d).

Much of the northern sector of the study zone was formerly occupied by the *Hacienda* Tenorio property; according to Sequeira's (1985) map, by 1880 this included the lands between the Corobicí and Tenorio Rivers north to the upper slopes of the Tenorio and Miravalles volcanoes. The *hacienda* was founded by 1770 (Gudmundson, 1983), when it was described in state records as the *sitio* Tenorio, a designation that implies grazing activity. By 1794, at least 1000 cattle were located in the property (Gudmundson, 1983). Colegial (1989) reported that, when taken over by the United Fruit Company in 1949 (for production of mules for use on banana plantations), only a few patches of the original forest had been cleared by small farmers for pastures, coffee and cane (the presence of smallholders has also been recorded by Edelman (1992)). However, records cited by Gudmundson (1983), indicating that 7000 of the total 17000 ha had been cleared by 1935, suggest that Colegial's more anecdotal account underestimated the amount of land clearance prior to acquisition by the United Fruit Company. For present purposes, the situation is confused further because much of the *Hacienda* Tenorio lies outside the study zone, i.e. the medium to upper slopes of the volcanoes. It seems likely that historic grazing would have been concentrated in the southern sectors, which are closer to the historic cattle-drivers' road to the Costa Rican Central Valley and contiguous with the other known cattle ranches. This is supported by the designation on the 1956 map series (IGCR 1956d) of a large wooded area of what is now the La Pacífica farm as 'Potrero [*i.e.*, pasture] La Pacífica', suggesting a traditional wooded *sitio*. The1945-based map series offers no guidance with respect to the northern zone itself, as this area was not covered by this map series.

Quantitative analysis

Methods

For much of the study zone, forest cover before the cattle-boom of the 1950s is documented in the 1:25000 map series published in 1950-1951, based on aerial photos taken in 1945 (IGCR 1955, IGCR 1956a, 1956b, 1956c, 1956d). However, the zone to the north of 10°30'N and east of 85°07'W, corresponding roughly with the northern sector of the study zone, was not covered by this series. However, this zone is included in the first 1:50000 map series, based on photos taken from 1945-1961. The state of forest cover in the study zone in 1945 (southern zone) and 1961 (northern zone) is characterized here based on random point sampling of these map series. Each sample point was classified as forest, swamp, deforested land (i.e. non-swamp land no longer under forest), and other land (roads, rivers, lakes, etc.) and for its location relative to the nearest watercourse (i.e. whether within 100m distance). Estimated proportional land cover by class was estimated as the proportion of all points falling in each class. Two-by-two contingency tables for presence of forest in relation to distance from nearest watercourse (i.e. whether within 100m) were compiled. Equality of ratios (forested/deforested) in the two distance classes was tested using Fisher's exact test; the odds ratio for forest cover in the two watercourse-distance classes and the associated confidence intervals were also estimated (Ramsey and Schafer, 1997). SPSS was used for both procedures (SPSS, 1996).

Current forest cover was characterized based on a set of 1:10000 aerial photographs taken in 1998. A sheet with 20 systematically spaced punch-holes was placed over each photo (each photo corresponds to an area of approximately 5.3 km^2), and four of these were randomly selected for location of random point samples at their centres. At each sample point, land cover was classified as either riparian forest, non-riparian forest, pastureland, agricultural land, scrub, swamp and other use (aquatic, public utilities, roads, urban zones and areas which could not be classified). In addition, for sample points which fell on unforested areas, the presence of individual trees in a circle of approximate area 0.25ha centred on the sample point was scored (1=one or more trees present, 0=absent). This area, although arbitrary in that it was derived from the scaled-up area of the punch-circles drawn on the photos, proved satisfactory in order to detect differences in tree stocking between matrix types. In addition, the presence of continuous forest within 100m of the sample point was scored. Sample points were also scored for their location relative to the nearest watercourse, as for the map data. The same analysis approach was used as for the map data. As field observations suggested that pastureland and proximity to watercourses both had positive effects on forest or tree presence, one-tailed probability values were used.

Results

In 1945, the southern sector of the study zone was close to 30 per cent forested (Figure 3-3a). By 1998, this proportion had fallen to 13.4 per cent (Figure 3-3c), of which almost half (6.7 per cent of total) consisted of riparian remnants. In 1945, points within 100m of watercourses were estimated to be almost three times more likely to be forested (Table 3-1). In 1998, points within 100m of watercourses were estimated to be more than 5 times as likely to be forested (Table 3-2). In addition, locations in pastureland are significantly more likely to have individual trees within 0.25ha surrounding area and to be within 100m of forest (Tables 3-3, 3-4).

In the case of the northern sector of the study zone, forest cover estimate for the 1945-1961 period (15.0 per cent) was lower than the 1998 estimate (24.2 per cent). In the case of the earlier period, there was no significant effect of proximity to watercourse on forest cover (Table 3-1). In the case of the 1998 data, locations within 100m of a watercourse were almost three times more likely to be forested (Table 3-2). Meaningful comparisons between pastureland and agricultural land were not possible for this sector because of the low proportion (2.0 per cent) of agricultural land.

Discussion (quantitative analysis)

The recent forest history of the study zone appears to diverge in several ways from the general trends for Guanacaste. Although there was considerable deforestation in the southern part of the study zone in the post-1945 period, this cannot be considered to more significant than previous deforestation, as the zone was already substantially deforested by this time. Deforestation was certainly rapid during this period, and probably more rapid than at any other time, but was not concentrated during this period. The southern sector also appears to be atypical in that post-1950s deforestation was associated at least as much with industrial agriculture as with cattle expansion. A large area of the study zone around the low hills known as Las Lomas, on the Hacienda Taboga, was deforested sometime after 1956, apparently to feed the new sugar-mill established on the farm in 1958 (Edelman, 1992) (Figure 3-4). The La Pacífica farm appears to have been deforested in the same period: as mentioned above, much of it appears as the wooded 'Potrero La Pacífica' on the 1945 map series. In aerial photos dating from 1956, it consists of mixed open and closed woodland, but by 1986 had been largely deforested. Currently, the farm is a mosaic of pastureland, forest remnants, shelterbelts and croplans (Jiménez *et al.*, 1987).

In the case of the northern section of the study zone, the foregoing analysis suggests an increase in forest cover following the 1945-61 period. Although it is possible that this is partially associated with mapping criteria discrepancies, (e.g. similar to those mentioned by Sader and Joyce, 1988) it probably also reflects abandonment or partial abandonment of marginal farms, e.g. in the hilly zone between Cañas and Palmira (Figure 3-2), where there are relatively large areas of young secondary forest (*tacotal*).

The implications of deforestation patterns as revealed both by the foregoing analysis and suggested by historical trends are considered below.

GENERAL DISCUSSION

Evidently, forest in the study zone has been profoundly affected by human intervention since the Spanish conquest. Although the most marked changes are likely to have occurred during the acceleration of deforestation from the late 19th century, it should not be assumed that the forest grazing practised before this time was harmless. Judging from the appearance of traditional *sitios* such as that pictured in Becker (1943), it seems to have radically transformed the forest. Grazing within forest has been identified as one forest practice expected to affect genetic processes (Namkoong *et al.*, 1996). Specifically, the latter authors comment that 'grazing would be expected to have a thinning effect on regenerating vegetation and hence could affect genetic drift. It could also affect the understorey vegetation that may also be directly grazed and hence exert a selection effect and changes in the population density of those species, affecting their selection and drift effects. Since grazing may also compact soils and alter stand structure, it may also induce selectively significant environmental changes'. It seems unlikely that wild ungulates would have any comparable effect, both because of smaller body size (less compaction) and lower population densities.

Such effects would not be expected to impact all species in the same way. More palatable species, and those with seeds less able to survive ingestion by cattle, would be expected to be most subject to reduction of population density. At one extreme, population fragmentation, as well as density reduction, may have occurred, as susceptible species disappeared from sites with severe grazing pressure. Recent studies suggest that probable consequences of population density reduction go beyond the selection and drift effects identified above, as reduction in population density of forest trees may affect pollinator behaviour. For example, in the tropical tree *Shorea siamensis*, Ghazoul *et al.* (1998) found that the proportion of pollinator flights between (as opposed to within) plants was positively correlated with increasing plant density. In fully or partially self-compatible species, such changes in pollinator behaviour may lead to increases in selfing rates. Thus, Murawski and Hamrick (1992) established that outcrossing rate in *Cavanillesia platanifolia* is density-dependent: isolated trees had high selfing, whereas trees in groups were predominantly outcrossed. Increased selfing rates are likely to lead to inbreeding depression, as in the case of the Madagascan endemic *Dombeya acutangula* ssp. *acutangula*.

(Gigord *et al.*, 1998). In self-incompatible species, decreased interplant movements may result in selection pressure in favour of increased self-compatibility (Murcia, 1996), with similar long-term consequences as for self-compatible species.

From the late nineteenth century, forest change in the study zone seems to have been typified by removal and fragmentation as well as density reduction, as traditional *sitios* were converted to improved pastureland or to agriculture. The earlier intensification of deforestation - i.e., as compared to the post-1950s increase in other parts of Guanacaste Province - implies an earlier onset of problems associated with habitat fragmentation. The pattern of deforestation in the study zone, as revealed by the foregoing analyses, has implications for the seriousness of such problems. Firstly, land conversion to grazing - both by attrition in the long (post-)colonial phase, and in subsequent change to 'improved' pastureland - has been less destructive of trees and forest than conversion to industrial agriculture. The latter is associated with 'classic' matrix-island landscape (e.g. Figure 3-4), whereas grazing lands in the study zone have a far higher density of individual trees and a higher tendency to be close to forests, reflecting a more diverse landscape structure. As presence of intervening trees and lower mean distance to forest fragments is likely to reduce reproductive isolation, this implies that forest fragments located in pastureland are likely to be less reproductively isolated than forest fragments located in agricultural lands. This is of relevance to viability of forest fragments, as the genetically homogenizing effects of random genetic drift can be mitigated by relatively low amounts of immigration (Hartl and Clark, 1989; Mills and Allendorf, 1996). Furthermore, small, isolated fragments may be subject to pollinator limitation of reproductive output (Murcia, 1996; Groom 1998; Jennersten, 1998). However, it is worth noting that the apparent relative benignancy of the pastureland matrix is likely to be a transient phenomenon without active human intervention to maintain current stocking levels. Current pasture trees are remnants of previous forests or more heavily wooded *sitios* and recruitment in pastureland appears to be virtually nil.

Secondly, deforestation and fragmentation in the study zone has clearly been a spatially non-random process. In both sectors of the study zone, proximity to watercourses is associated with significantly and notably higher probabilities of forest persistence. Both non-randomness as such and the particular 'bias' in question have important genetic implications. A number of species native to the study zone occur in both riparian and non-riparian situations, e.g. *Pithecellobium saman*, *Anacardium excelsum*, *Astronium graveolens*, *Bombacopsis quinata*, *Enterolobium cyclocarpum*, *Spondias mombin*, *Swietenia macrophylla*. It is possible that the marked environmental differences between riparian and non-riparian forest in the study zone (Janzen, 1976) could have given rise to local adaptation, implying greater than expected loss in genetic diversity than under random deforestation (Young *et al.*, 1996). It is also possible that deforestation may have led to reduction in gene flow between riparian and non-riparian remnants, thereby favouring local genetic differentiation. This outcome might lead to higher overall diversity, but at the cost of loss of some populations and increased isolation of remnants.

A 'bias' in forest retention in favour of riparian types implies a change in the spatial characteristics of forest in the study zone, i.e. a redimensioning from two dimensions to one. This is of particular relevance to genetic impacts of forest fragmentation. Although, as indicated above, theory predicts that only a small number of immigrants are needed to offset the genetically homogenizing effects of random genetic drift, this conclusion applies to a two-dimensional configuration of populations. The same body of theory predicts that configurations tending more to unidimensionality, such as population fragments located on essentially linear landscape elements such as ridge-tops and watercourses, will be more prone to population subdivision and associated inbreeding effects than two dimensional configurations. Under such conditions, considerably more gene flow per generation is required to avoid accumulation of relatedness in subpopulations (Hartl and Clark, 1989; Wright, 1969). For example, under the Stepping Stone migration model (which assumes that gene flow occurs predominantly between adjacent populations) and with migration rates of 0.1 and $(2)(10^{-5})$ respectively for adjacent and long-distance gene flow, 'considerable' local differentiation will occur if $N_e<100$ (Kimura and Weiss, 1964). Consequently, such populations are expected to be more susceptible to population fragmentation and isolation than those located in two-dimensional configurations. The prevalence and special characteristics of riparian remnants suggest that they should be taken into account in studies of genetic and other effects of forest fragmentation.

The higher probability of forest persistence close to watercourses may to some extent be attributable to legal protection. Current Costa Rican law prohibits logging within 15m of rural watercourses, or within 50m if on sloping ground. (Costa Rica Legislative Assembly 1996). However, this law has been widely violated, with virtual impunity (Porras and Villareal, 1993; Campos *et al.*, 2001) and, in the study zone, has manifestly been ignored in many cases. Furthermore, that many riparian strips extend beyond even the higher 50m legal minimum suggests that other, non-legal factors are of at least equal importance. Riparian forest is likely to be intrinsically less susceptible to deforestation, either because it is less susceptible to fire, or (in some cases) because steep topography may make it useless for agriculture or inaccessible and dangerous to livestock. Additionally, landowners may themselves recognize the usefulness of riparian forest in protection of watercourses and, in some cases, provision of dry season grazing which, at least in the medium-term, may be compatible with forest persistence.

In itself, the continued presence and greater robustness of riparian forest is encouraging, as the essentially permanent nature of the causal factors mentioned above suggest that such forest may persist as a long-term landscape element. However, such persistence is also dependent on biological viability. Although the occurrence of ancient, naturally isolated gallery forest remnants, *e.g.* in the *llanos* of Colombia and upland Belize (Kellman *et al.*, 1994), indicates that that these can be viable in the long-term, this conclusion is not necessarily applicable to narrower, anthropogenic fragments.

The present study suggests Guanacastecan forest cover notably higher than reported in other studies. For example, according to Sader and Joyce (1988), virtually the whole of lowland Guanacaste had been deforested by 1940, whereas Sánchez-Azofeifa *et al.* (2001) reported 3 per cent forest cover (i.e. 284km^2) for the Province. These discrepancies reflect various factors, particularly the resolution of the imagery used and stated differences in approach. Sader and Joyce's methods did not permit detection of forest fragments smaller than 55ha. Sánchez-Azofeifa *et al.*'s methodology permitted much higher resolution (3ha), but, according to the authors, was unsuited to detection of tropical dry forest. However, even non-detection only of fragments <3ha, as well as linear fragments of small width but large total areas, is likely to result in estimates of total forest cover which fail to take into account biologically significant and visually prominent landscape elements. These considerations suggest that the study zone is less atypical of the rest of Guanacaste than casual comparison with these other studies might suggest. Rather, the landscape elements and features of the study zone seem similar to much of lowland Guanacaste, and suggest that many of the conclusions drawn above are likely to have applicability beyond the study zone. Riparian remnants, particularly, persist throughout seasonally dry Central America, even in some of the most highly deforested zones. Furthermore, this tendency seems to extend beyond the region: Kellman *et al.* (1996) remark that 'gallery forests are by far the most frequent form of natural forest patch in subhumid tropical landscapes', a phenomenon probably largely explicable in many zones by the same combination of factors mentioned above. Such remnants would appear to have an important role to play in biodiversity conservation, particularly outside protected areas, where forest may be otherwise scarce.

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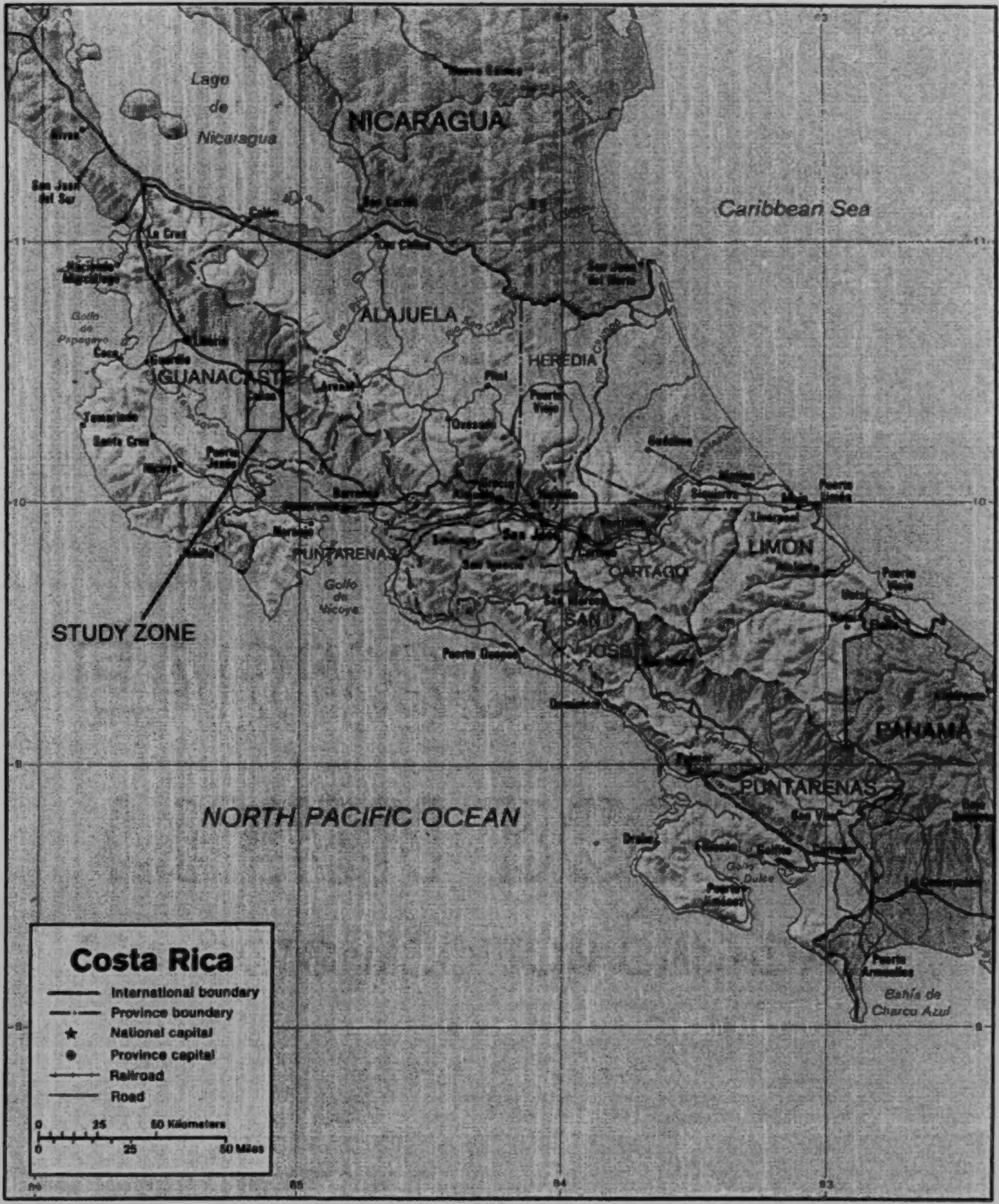
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Santa Cruz
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PUNTARENAS
Golfo de Nicoya
STUDY ZONE
San José
CARTAGO
LIMON
SAN JOSE
Puerto Quepos
PANAMA
PUNTARENAS
Golfito
Golfo Dulce
Puerto Jiménez
Puerto Armuelles
Bahía de Charco Azul
NORTH PACIFIC OCEAN
Costa Rica
International boundary
Province boundary
National capital
Province capital
Railroad
Road
0 25 50 Kilometers
0 25 50 Miles
85
84
83
11
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8

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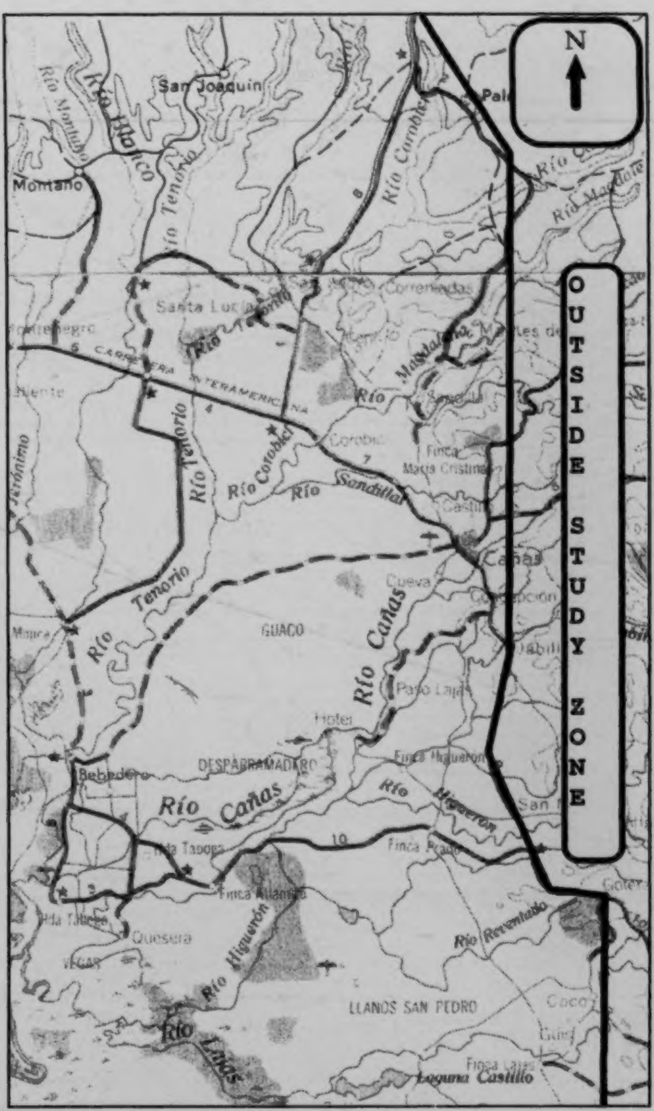
Figure 3-1. Costa Rica: major cities, relief and location of the study zone (Source: Central Intelligence Agency (1987); public domain material).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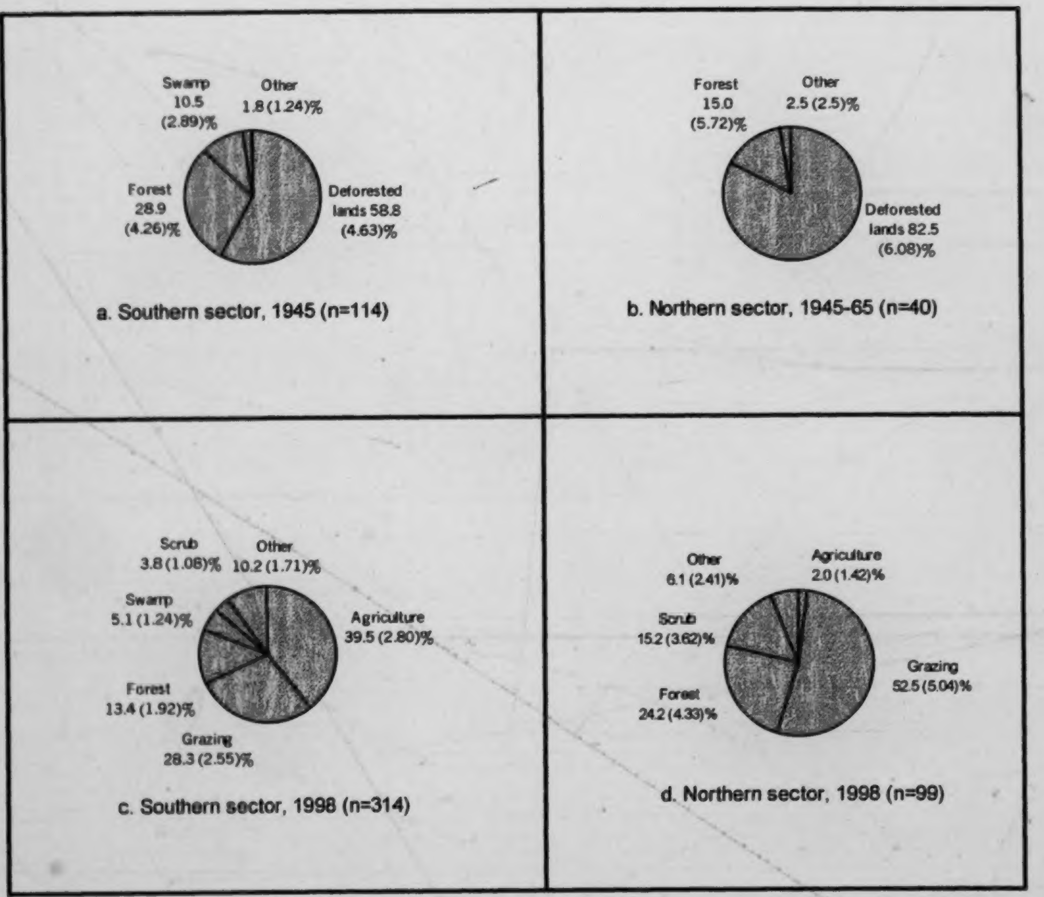
San Joaquin
Río Montano
Río Tenorio
Río Corobicí
Montano
Santa Lucía
Río Tenorito
Correntadas
CARRETERA INTERAMERICANA
Río Magdalena
Corobicí
Finca María Cristina
Río Sandillal
Cañas
Cueva
GUACO
Río Cañas
Paso Lajas
Hotel
Bebedero
DESPARRAMADERO
Río Higuerón
Finca Higuerón
Finca Prado
Quesera
Río Reventado
LLANOS SAN PEDRO
Río Lajas
Laguna Castillo

Figure 3-2. The study zone (scale: 1:200000). Shading (on original) indicates wooded areas, but does not include all such areas (source: IGN 1988a, 1988b, reproduced by permission (see Appendix 4)).



a. Southern sector, 1945 (n=114)

b. Northern sector, 1945-65 (n=40)

c. Southern sector, 1998 (n=314)

d. Northern sector, 1998 (n=99)

Figure 3-3. Land use in the study zone, 1945-1998: estimated percentage cover (standard error) by use category.

Figure 3-4. Las Lomas and Llanos de San Pedro area, Hacienda Taboga, in 1956 (left) and 1986 (right) (photography: Instituto Geográfico Nacional (Costa Rica)

Table 3-1. Two-way classification of sample points by distance to nearest watercourse (≤100m, >100m) and presence of forest in 1945 (southern sector) and 1945-61 (northern sector)

SOUTHERN SECTOR

	forest cover		
	unforested	forested	totals
distance to nearest watercourse			
≤100m	11	12	23
>100m	56	21	77
totals	67	33	100

probability[1] (one-tailed) (Fisher's exact test): 0.026

odds ratio[2] (95% confidence limits) of forest cover for distance ≤100m:distance >100m: 2.909 (1.114-7.595)

NORTHERN SECTOR

	forest cover		
	unforested	forested	totals
distance to nearest watercourse			
≤100m	6	1	7
>100m	27	5	32
totals	33	6	39

probability[1] (one-tailed) (Fisher's exact test): 0.999

odds ratio[2] (95% confidence limits) of forest cover for distance ≤100m:distance >100m: 0.900 (0.088-9.178)

---

[1] probability of observed values under null hypothesis of no association between forest cover and distance to nearest watercourse; [2] where sample odds for each distance class = (p/1-p) (p=proportion for points that are forested)

Table 3-2. Two-way classification of sample points by distance to nearest watercourse (≤100m, >100m) and presence of forest in 1998

SOUTHERN SECTOR

	forest cover		
	unforested	forested	totals
distance to nearest watercourse			
≤100m	44	23	67
>100m	181	18	199
totals	225	41	266

probability[1] (one-tailed) (Fisher's exact test): <0.001

odds ratio[2] (95% confidence limits) of forest cover for distance ≤100m:distance >100m: 5.256 (2.612-10.577)

NORTHERN SECTOR

	forest cover		
	unforested	forested	totals
distance to nearest watercourse			
≤100m	12	9	21
>100m	57	15	72
totals	69	24	93

probability[1] (one-tailed) (Fisher's exact test): 0.044

odds ratio[2] (95% confidence limits) of forest cover for distance ≤100m:distance >100m: 2.850 (1.013-8.020)

---

[1] probability of observed values under null hypothesis of no association between forest cover and distance to nearest watercourse; [2] where sample odds for each distance class = (p/1-p) (p=proportion for points that are forested)

Table 3-3. Two-way classification of sample points by land use (agriculture, pastureland) and presence of trees within radius of 27.5m,1998

SOUTHERN SECTOR

	presence of trees within 27.5m		
	present	absent	totals
land use			
agriculture	27	97	124
pastureland	62	27	89
totals	89	124	213

probability[1] (one-tailed) (Fisher's exact test): <0.001

odds ratio[2] (95% confidence limits) of tree presence for pastureland:agriculture: 8.250 (4.432-15.357)

NORTHERN SECTOR

	presence of trees within 27.5m		
	present	absent	totals
land use			
agriculture	1	1	2
pastureland	36	16	52
totals	37	17	54

probability[1] (one-tailed) (Fisher's exact test): 0.535

odds ratio[2] (95% confidence limits) of tree presence for pastureland:agriculture: 2.25 (0.132-38.27)

---

[1] probability of observed values under null hypothesis of no association between land use and tree presence; [2] where sample odds for each distance class = (p/1-p) (p=proportion for points that are forested)

Table 3-4. Two-way classification of sample points by land use (agriculture, pastureland) and presence of forest within 100m

SOUTHERN SECTOR

	distance to nearest forest		
	≤100m	>100m	totals
land use			
agriculture	25	99	124
pastureland	41	48	89
totals	66	147	213

probability[1] (one-tailed) (Fisher's exact test): <0.001

odds ratio[2] (95% confidence limits) of forest presence for pastureland:agriculture: 3.383 (1.847-6.195)

NORTHERN SECTOR

	distance to nearest forest		
	≤100m	>100m	totals
land use			
agriculture	2	0	2
pastureland	19	33	52
totals	21	33	54

probability[1] (one-tailed) (Fisher's exact test): 0.147

odds ratio[2] (95% confidence limits) of forest presence for pastureland:agriculture: 1.105 (0.962-1.270)

---

[1] probability of observed values under null hypothesis of no association between land use and forest presence;
[2] where sample odds for each distance class = (p/1-p) (p=proportion for points that are forested)

Chapter 4

INHERITANCE, LINKAGE AND NEUTRALITY OF ALLOZYMES OF THE NEOTROPICAL TREE *ANACARDIUM EXCELSUM* (BERTERO & BALBIS EX KUNTH) SKEELS (ANACARDIACEAE)

INTRODUCTION

Anacardium excelsum (espavel, espavé, wild cashew) is a large, evergreen tree native from Honduras in northern Central America, south to Ecuador and the Guyanas (Hartshorn and Gentry, 1991). In seasonally-dry zones, as in the Pacific watershed of Central America, *A. excelsum* is generally found in moist sites less subject to seasonal drought, for example riparian forests. The population genetics of *A. excelsum* is of interest for two main reasons. Firstly, gallery forest appears to represent a large proportion of the remaining closed forest of this largely deforested zone (Chapter Three; Janzen, 1986) As a dominant and common species of such forest (Glander and Nisbett, 1996), the 'genetic health' of *A. excelsum* populations is linked to the conservation status of these habitats. Secondly, information about the population genetics of *A. excelsum* may also generate insights into conservation genetics of other taxa with similar life histories and similar patchy or fragmented distributions.

The suitability of allozymes as genetic markers for population genetics studies is partly due to their codominant inheritance. However, as various factors may cause apparent or real departures from codominance (Gillet and Hattemer, 1989), it should be demonstrated rather than simply assumed. In the present article, enzyme polymorphism and its genetic basis in six enzyme systems of *A. excelsum* are described. Linkage relationships and neutrality of these enzymes are also reported, as these characteristics affect to some degree their usefulness in population genetics studies, *e.g.* Ritland and Jain's (1981) algorithm for outcrossing rate estimation assumes that loci are independent and selectively neutral between gametic union and point of census. The use of five of these polymorphic loci in studies of population genetics of the species is reported elsewhere (Chapter Five).

MATERIALS AND METHODS

In April 1999, open-pollinated seeds were collected from 105 individual trees in 12 forest fragments located in the *cantón* of Cañas, Guanacaste Province, Costa Rica (Table 4-1). *A. excelsum* diaspores are both dispersed by bats, such as *Artibeus* spp. (Janzen *et al.*, 1976) and fall under gravity when ripe (drupes weigh up to 3g each). The collections were made from the ground below inflorescence-bearing branches of each tree, avoiding areas beneath overlapping crowns of neighbouring trees.

There appear to be no published enzyme extraction or electrophoresis protocols for *A. excelsum*. For this reason, combinations of different extraction buffers, electrophoresis buffer systems and enzymes were screened in order to identify potentially useful markers. The systems AK (adenylate kinase, E.C. number 2.7.4.3), LAP (leucine aminopeptidase, 3.4.11.1), MDH (malate dehydrogenase) (E.C. number 1.1.1.37), PGD (phosphogluconate dehydrogenase, 1.1.1.43), PGM (phosphoglucomutase, 5.3.1.9) and UGPU (UTP-glucose-1-phosphate uridylyltransferase, 2.7.7.9) were selected based on their resolution or consistency with expected quaternary structure. Other enzyme systems were rejected for various reasons: because no activity was detected (alcohol dehydrogenase leaf tissue; β-galactosidase, α-glycerophosphate dehydrogenase, glycerate-2-dehydrogenase, glutamic dehydrogenase, sorbitol dehydrogenase, succinate dehydrogenase, xanthine dehydrogenase), because staining resolution or intensity was unsatisfactory on all tested buffer combinations (aconitase, acid phosphatase, aldolase, diaphorase, fumarase, glucose dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, isocitric dehydrogenase, malic enzyme, mannose-6-phophate isomerase, peroxidase), because acceptable resolution or intensity was only inconsistently achieved (esterase), because of interpretation difficulties (superoxide dismutase) or because polymorphy was not detected (alcohol dehydrogenase radicular tissue, catalase, glucose-6-phosphate-dehydrogenase, hexokinase, glucose-phosphate isomerase, shikimic kinase, meniadone reductionase).

The seeds were germinated in greenhouse facilities of the University of Alberta and enzyme extracts prepared by grinding fresh, flash-frozen, leaf disks in Liengsiri *et al.*'s (1990) extraction buffer #9. The homogenate was absorbed on Whatman #3 filter paper wicks, and stored at -80°C until needed. Horizontal starch (Connaught Laboratories, Ontario) gel electrophoreses were carried out using a pH5.7 histidine-citrate buffer system (Wendel and Weeden, 1990). Allozymes were visualized using staining protocols from Hodgkiss (2001), Liengsiri *et al.* (1990) and Wendel and Weeden (1990). Extraction, grinding and electrophoretic techniques are described in detail elsewhere (Appendix One). The isozymatically invariable *Pinus resinosa* Ait. (Fowler and Morris, 1977) was used as a control. Loci were named in ascending numerical order according to their mobility, whereas alleles of each locus were assigned alphabetic codes, 'A' representing the most common allele.

Inheritance

Analysis of segregation ratios followed the methodology outlined by Gillet and Hattemer (1989). Two specific null hypotheses were tested: that of 1:1 ratios of heterozygous and homozygous progeny of heterozygous mother-trees (*e.g.* $n_{AB} = n_{AA} + n_{BB}$ for mother-tree AB) and that of 1:1 ratios of progeny genotypes AC and BC in progeny of AB-heterozygous mother-trees (LAP only). The binomial probabilities of observed ratios were calculated. Subsequently, the significance of departure from 1:1 of individual arrays was examined using the Dunn-Šidák method of sequential Bonferroni testing (Sokal and Rohlf, 1995). Under this procedure, the ascendingly ranked probabilities are compared sequentially to a steadily increasing critical value $1-(1-\alpha)^{1/n}$, where α is the chosen probability value (0.05 in this case) and n is the number of arrays not already tested; testing continues until the first nonsignificant array is detected. The significance of departure from 1:1 of the pooled segregation ratios for each enzyme was tested using the *G*-test with Williams's adjustment (Sokal and Rohlf, 1995).

Formally, Gillet and Hattemer's methodology requires independent parental genotyping, that is, use of parental germplasm. When parental material is unavailable, as in the present case, putatively heterozygous mother-trees must first be identified on the basis of the presence of two homozygote types in their progeny. However, this means of identifying heterozygous parents for itself implicitly assumes codominant inheritance, because only under codominant inheritance can all homozygotes be distinguished from heterozygotes. Therefore, the strongest formal conclusion that can be made is that the segregation ratios of the observed phenotypes are consistent with their proposed genetic interpretations. However, when phenotypes are consistent with the quaternary structure of the enzyme in question, and particularly when apparent heterozygotes are detected, the degree of confidence attached to as assertion of codominant inheritance on the basis of the progeny segregation does not appear to be critically less than when parental material is used.

Additional analyses of segregation ratios were suggested by two aspects of the results. Firstly, there was a tendency towards homozygote excess in progeny arrays of putative heterozygotes, particularly of AK*2* and PGD. Secondly, three homozygote types were also observed in two of the LAP arrays. Both phenomena could occur as a result of inclusion in putative progeny arrays of seed not produced by the mother-tree in question; although many *A. excelsum* drupes fall directly from the tree, it is nevertheless possible that those collected from beneath any tree may include extraneous seed dropped by bats or, less commonly, by howler monkeys (Glander, 1979). Inclusion of extraneous seed in individual progeny arrays could contribute to the observed homozygote excess via two mechanisms. Firstly, >1 homozygote types could occur in progeny arrays of homozygote mothers, leading to incorrect assignment of heterozygous maternal genotypes. This, in turn, is likely to produce apparent segregation distortion. For example, for a diallelic locus with allele frequencies $p_A=0.8$ and $q_B=0.2$, the expected proportions of AA and AB genotypes in progeny of AA homozygotes are 0.8 and 0.2, respectively. In such a case, the inclusion of an extraneous BB 'progeny', whilst leading to redesignation of maternal genotype to AB, would have little effect on the ratio of homozygotes to heterozygotes. Consequently, segregation ratio would be approximately 4:1 instead of the 'expected' 1:1. Secondly, segregation ratios of genuinely heterozygous individuals will depart from 1:1, the magnitude and direction of distortion depending on the admixture rate and the genotype frequencies in admixed seed.

As some applications, e.g. mating system analysis, are dependent on the legitimacy of progeny arrays, the possibility that arrays may contain extraneous seed is of interest and was investigated further. If the actual progeny arrays of heterozygous trees have, as expected, equal proportions of heterozygotes and homozygotes, and if admixed seed is drawn randomly from the population as a whole, then, for diallelic loci, the proportion of extraneous seed is:

$$p_e = \frac{f\left(XX_{(XY)}\right) - 0.5}{f\left(XX_{pop}\right) - 0.5} \qquad \text{...(Equation 4.1)}$$

where $f\left(XX_{(XY)}\right)$ = observed frequency of homozygotes (or heterozygotes, in case of greater numbers of heterozygotes in the population as a whole) in progeny arrays of putative heterozygotes and $f\left(XX_{(pop)}\right)$ = frequency of homozygotes (heterozygotes) in the population as a whole.

The parameter p_e was estimated for AK2, LAP, PGD, PGM and UGPU for five populations (Table 4-2). Populations in which there were <3 putatively heterozygous trees were excluded, as were cases in which total number of trees sampled was ≤5, as in these cases the ≥3 putatively heterozygous trees would themselves largely determine the estimated populational genotypic frequencies. The triallelic locus LAP was included as, in the five populations, observed frequencies of AC, BC and CC genotypes were sufficiently small as not to cause appreciable bias in the use of the above equation. As the equation applies to progeny of heterozygous trees only, individual arrays in which homozygote excess appeared most likely to be caused by incorrect designation of maternal homozygotes as heterozygotes were not included (exceptions are noted in Table 4-2).

Generalized seed admixture would be expected to be reflected in a positive relationship between denominator and numerator of Equation 4.1. The correlation coefficient between these two variables was therefore estimated. Finally, the effect on mating system parameter estimation of inclusion of extraneous seed in progeny arrays was explored by simulation. Simulated sample data sets with known allele frequencies (3 loci), multilocus outcrossing rate (t_m) and maternal inbreeding coefficient F were generated using Ritland's 'datagen' and 'convdata' programmes (Ritland, 1996). Each data set consisted of 20 progeny of each 20 parents. A 'seed pool' data set was also formed by pooling all family arrays and randomizing by multilocus genotype or 'seed'. A programme was then written in Visual Basic for Excel in order to simulate seed movement into the sample arrays from the 'seed pool', as follows. Firstly, the number of sample trees (N) to be affected by the inward migration, and a fixed number (e) of extraneous seed per array were specified. For each run, the sample trees to be affected were randomly chosen and, for each of them, e of their 'real' progeny were overwritten by e multilocus genotypes randomly chosen from the 'seed pool' data set. Values of maternal inbreeding coefficient (F) and t_m (multilocus outcrossing rate) were then recalculated using

Ritland's MLTR programme (Ritland, 1996). The simulation programme was run for two pre-admixture t_m settings ($t_m \approx 1.0$ and $t_m \approx 0.7$). Within each t_m, runs were made for three allele frequency scenarios. For each allele frequency scenario, five runs for each of four seed movement scenarios were made: general, intense seed movement (*i.e.* all 20 trees affected, 50 per cent extraneous composition in each array); general but moderate movement (20 trees affected, 10 per cent extraneous composition), sporadic but intense (2 trees, 50 per cent), and sporadic and moderate (2 trees, 10 per cent).

Linkage disquilibrium

As neither haploid material nor progeny-test data were available, linkage disequilibrium coefficients based on frequencies of coupling and repulsion heterozygotes were not estimable. Therefore, values of Burrows's composite linkage disequilibrium coefficient Δ_{ij} and the corresponding correlation coefficient (Weir, 1979) were estimated for each of the 12 populations. Significant values of Δ_{ij} imply that there are differential frequencies of coupling and repulsion heterozygotes and/or non-random union of gametes (Roberds and Brotschol, 1985). Ohta's (1982) multiple population linkage disequilibrium (D) coefficients were also estimated. Ohta's partitioning of the variance of linkage disequilibrium was devised principally to distinguish between epistatic selection and genetic drift as causes of observed disequilibria: when D_{IS}^2 (the variance of disequilibrium within populations) is less than D_{ST}^2 (the variance of correlation between different gametes of one subpopulation relative to that of the total population) and D'^2_{IS} (variance of within gamete correlation in a subpopulation relative to the total population) is greater than D'^2_{ST} (variance of disequilibrium of the total population), then population subdivision (*i.e.* genetic drift) rather than selection is likely to be the main cause of linkage disequilibria (Kremer and Zanetto, 1997; Ohta, 1982). In the case of the triallelic locus LAP, for estimation of both Burrows's and Ohta's coefficients all alleles except the most common were pooled to a synthetic allele (Kremer and Zanetto, 1997). Missing values were eliminated from the data set as these add no information on non-gametic or gametic correlations in allele frequencies. POPGENE (Yeh and Boyle, 1997) was used for estimation of all the above disequilibrium parameters.

MDH and AK*1* were omitted from the above linkage analysis due to interpretation difficulties (see below). However, in the case of AK*1*, an apparent linkage with PGD was tested for by chi-square analysis of a two-by-three contingency table of AK*1* phenotypes (*i.e.* presence or absence of most frequent band, see inheritance results, below) against PGD putative genotypes.

Neutrality

POPGENE was also used to test for selective neutrality. It uses Stewart's algorithm for the Ewens-Watterson neutrality test, as detailed in Fuerst et al. (1977) and Manly (1985). One thousand iterations were employed for generation of simulated distributions of expected values of the F statistic, *i.e.* the sum of squared allele frequencies under the null hypothesis, under which the configuration of alleles corresponds to the equilibrium values for the infinite alleles mutation model (Hartl and Clark, 1989). AK*1* and MDH were omitted from the neutrality analysis, for the same reasons indicated above. Neutrality was tested by population and for pooled data.

RESULTS

Inheritance

Adenylate kinase

The simplest zymograms were characterized by the presence of two invariable bands of unequal intensity, suggesting the presence of two loci (Figure 4-1). As neither band was always present, it was concluded that both loci are polymorphic. When the putative allele AK*2*-A was absent, there was always a band cathodal to putative allele AK*1*-A, indicating an overlap in the range of activity of the two loci (Figure 4-2). Other putative AK*2* alleles could not be identified, although the possibility of a 'hidden' allele of equal mobility to AK*1*-A cannot be discounted. The pooled segregation ratio for AK*2* (Table 4-3) shows a highly significant homozygote excess. One array (El Cepo 1554) had a probability value smaller than its Dunn-Šidák critical value (0.002, n=26) (Table 4-4), i.e. departed significantly from the expectation under codominance.

Scoring of AK*1* was impeded both by inconsistent resolution and the apparent overlap with AK*2*. Consequently, segregation ratios could not be determined.

Leucine aminopeptidase

One locus was detected. It followed the expected (May, 1998) monomeric banding pattern. Three putative alleles were observed (Figure 4-3). Overall segregation ratios were not significantly different from expectations for any of the pooled LAP data sets (Table 4-3). The binomial probability of the segregation ratio of one array (Marcela-419) was less than its corresponding Dunn-Šidák value (0.0014, n=36) (Table 4-5), i.e. departed significantly from the expectation under codominance. Two progeny arrays (El Ojoche-19, Toronja-107) each contained all three homozygous types.

Malate dehydrogenase

Malate dehydrogenase zymograms generally consisted of an invariant pattern with five clear bands. An occasional variant four-banded phenotype was observed (Figure 4-4). As there was no clear genetic interpretation (see discussion), no segregation analysis was made.

Phosphogluconate dehydrogenase

PGD zymograms showed two loci, one of them (PGD*2*) too indistinct to score. PGD*1* displayed the expected dimeric banding pattern, with two loci (Figure 4-5). The putative BB homozygote in general showed two additional faint bands anodal of the main band (Figure 4-5). Although the origin of these bands is not clear, they are easily distinguished from the heterodimer and AA homodimer bands of the putative heterozygote on the basis of their more anodal positions and lower intensities.

The pooled segregation ratio for PGD*1* (Table 4-3) shows a highly significant homozygote excess. Two arrays (El Cepo 1511, El Cepo 1566) had probability values smaller than their respective Dunn-Šidák critical values (0.0015, n=33; 0.0016, n=32), i.e. departed significantly from the expectation under codominance (Table 4-6).

Phosphoglucomutase

PGM is a monomeric enzyme (May, 1998), typically with 2 isozymes (Weeden and Wendel, 1990). Consistent with expectations, the PGM zymograms show two loci, one of them (PGM*1*) clearly resolved. This locus showed two single-band phenotypes and one double-banded phenotype, scored as two homozygotic types and the corresponding heterozygote (Figure 4-6). Faint, presumably artifactual, banding in the putative B-allele position was generally observed in putative AA genotypes (Figure 4-6). The pooled number of homozygotes was not significantly different from the number of heterozygotes (Table 4-3). The binomial probabilility of the most divergent ratio (El Cepo-1549) was greater than its corresponding Dunn-Šidák critical value (0.0013, n=40) (Table 4-7), i.e. did not differ significantly from expectations under codominance.

UTP-glucose-1-phosphate uridylyltransferase

The zymograms show two loci, one of which (UGPU*2*) was polymorphic (Figure 4-7). The observed banding patterns correspond to expectations for this reportedly monomeric (Chase *et al.*, 1995), *i.e.* one or two bands per lane (Figure 4-7). The number of homozygotes was not significantly different from the number of heterozygotes (Table 4-3). The probabilility of the most divergent ratio (R.S.R.-5, p=0.01) was greater than its corresponding Dunn-Šidák critical value (0.0024, n=21) (Table 4-8), i.e. did not differ significantly from expectations under codominance.

Incidence and effect of seed admixture

Estimates of p_e (proportion of admixed seed) for AK*2*, LAP, PGD, PGM and UGPU for five populations are detailed in Table 4-9. Ratios of homozygotes to heterozygotes in the general population and the collections from putative heterozygotes were of the same sign in nine cases and opposite sign in 7 cases. The correlation between denominator and numerator in the seed admixture was positive but of questionable significance (r=0.32, one-tailed probability = 0.12). Six of the nine positive estimates of the proportion of admixed seed were between 0.11 and 0.23. There was one estimate >1, found in the only case where both ratios showed heterozygote excess. The other two relatively high estimates of proportion of extraneous seed derive from the Congojas population, but estimates for two other loci for the same population were negative.

In the simulation, only general, intense seed movement (*i.e.* all 20 trees affected, 50 per cent extraneous composition in each array), particularly for $t_m \approx 0.7$, led to marked overestimation of t_m and gross underestimation of inbreeding coefficients (see cases 2.1-2.3, Table 4.10). Other movement scenarios (general but moderate movement (N=20, e=2), sporadic but intense (N=2, e=0.5), and sporadic and moderate (N=*2*, e=*2*)) had little effect on t_m estimates and intermediate effect on maternal *F*. In several cases, there were three homozygote types for the triallelic locus in one or more arrays, leading to aborted MLTR execution.

Linkage disequilibrium

There were significant ($p<0.05$) linkage disequilibria in 7 of the 120 permutations of 12 populations and 5 loci (Table 4-7). These included three cases of positive disequilibria between AK*2*-A and UGPU-A; overall, correlations (regardless of significance) between these loci were positive in ten of the 12 populations, and in no case negative. There were also three significant disequilibria between AK*2* and PGD; one of these was negative, but in general correlations between AK*2*-A and PGD-A were positive. Each of the other significant disequilibria were observed in one population only, with no evidence of consistent trends. The separate analysis of PGD-AK1 showed a strong, highly significant linkage (Figure 4-8, Table 4-12). For all pairwise locus combinations, D_{IS}^2 was less than D_{ST}^2 and $D'_{IS}{}^2$ was greater than $D'_{ST}{}^2$ (Table 4-13), suggesting that disequilibria are due to drift rather than selection.

Neutrality

Out of 60 locus and population combinations, there were six cases of significant departures from selective neutrality (observed *F* equal to or outside the 95 per cent confidence limits) (AK*2* in El Rodeo, LAP in Ojoche and Palmira, PGD and PGM in E.J.N., UGPU in Paso Hondo) (Table 4-14). On an individual population basis, no locus had observed *F* consistently close to upper or lower limits. However, PGM evinced significant selective non-neutrality in the case of the pooled data.

DISCUSSION

Inheritance

With the exception of MDH, the loci studied show banding patterns consistent with their expected quaternary structure. In particular, given this structure, and the putative homozygous genotypes observed for each, they exhibited those heterozygote patterns expected under codominant inheritance. There is no obvious interpretation for the MDH zymograms. *A priori*, a rare variant in otherwise invariable zymograms would be predicted to correspond to a heterozygous genotype. However, in this case the rare phenotype is simpler than the variant, *i.e.* has fewer bands, and even a tentative scoring as heterozygous seems unjustified.

In spite of the evidence of codominant inheritance, there appears nevertheless to be a tendency to homozygote excess, strongest in AK*2* and PGD. The presence of three homozygotes in two of the LAP arrays was also unexpected. As noted above, seed admixture represents one possible explanation for this phenomenon. However, evidence for the occurrence of this phenomenon is not convincing. In cases where opposite tendencies occur, the implication is that seed admixture is not responsible for observed homozygote excess, whilst the lack of significant correlation between numerator and denominator of Equation 4.1 and the occurrence of negative and positive estimates for different loci within populations suggests that admixture, insofar as it is occurring at all, is likely to be sporadic and concentrated on particular trees, *e.g.* trees on bat flight paths and/or close to feeding roosts. This hypothesis accords well with the pattern of homozygote excess in LAP, PGD and UGPU, which appears to be concentrated in specific arrays rather than generalized. In these loci, when arrays with nominally significant departures from 1:1 (*i.e.* probability of ≤ 0.05 under the null hypothesis of 1:1 segregation) are disregarded, the remaining progeny show similar numbers of homozygotes and heterozygotes (204:210, 237:212, 154:146, respectively) and the number of arrays with insignificant homozygote excess is similar to the number of arrays with insignificant heterozygote excess (13:15, 14:10, 9:7). Under generalized seed admixture, homozygote excess would be expected to predominate even after excluding individually significant arrays, as homozygous genotypes tend to be more common than heterozygous genotypes (*i.e.*, under panmixia, $p^2+q^2\geq 2pq$). In the case of AK*2*, on removal of individual nominally significant arrays homozygote excess is no longer significant, but remains marked (205:168) and there are more than twice as many positive as negative departures from 1:1 (14:6). As indicated above, in the case of AK*2*, the presence of an additional intermediate allele, masked by AK*1*, cannot be ruled out, and could be responsible for the observed homozygote excess. It should be noted, however, that there are other possible explanations for the homozygote excess, in these and the other loci, *e.g.* meiotic drive (Finkeldey, 1998) or gametic selection.

The simulation of effects on mating system estimates suggests that sporadic seed admixture of this type is unlikely to have a major effect on estimation of outcrossing rate.

Linkage disequilibrium

With 120 permutations of population and locus-pair, chance (*i.e.* type two error) would be expected to result in six estimates with associated probabilities of 0.05, and would appear to be the most parsimonious explanation for the observed disequilibria involving LAP, which have relatively high probabilities under the null hypothesis and/or inconsistency of sign in different populations.

The low probability values and high consistency of sign associated with the disequilibria between AK*2*-UGPU, AK*1*-PGD and, less convincingly, AK*2*-and PGD, make the above explanation less likely, and suggest appreciable linkage disequilibria in this tropical angiosperm, a result consistent with findings in temperate broadleaves (Granger, 1996; Huang *et al.*, 1996; Roberds and Brotschol, 1985; Zanetto *et al.*, 1996) and temperate and boreal conifer species (Cheliak and Pitel, 1985; Strauss and Conkle, 1986; Xie *et al.*, 1991; Yang and Yeh, 1993; Yeh *et al.*, 1994).

The lowest reported chromosome number for species in the *Anacardium* genus is $2n=24$ for *A. occidentale* (cashew) (Mitchell and Mori, 1987; Anonymous, 2002); there appear to be no specific reports for *A. excelsum* (Anonymous, 2002). Assuming that the loci on different chromosomes have equal chances of being selected for study, and assuming that *A. excelsum* has $2n=24$, then the probability of any two of the six loci (including AK*1*) belonging to the same linkage group is $p= 1-[(12)(11)(10)(9)(8)(7)= / 12^6] =0.77$, indicating that, *a priori*, it is relatively likely that physical linkage is responsible for at least one of the observed disequilibria.

Whether linkage is physical (*i.e.* location on the same chromosome) or non-physical, disequilibrium can be caused either by epistatic selection or genetic drift. The values of Ohta's coefficients (*i.e.* $D_{IS}^2 < D_{ST}^2$, $D'_{IS}{}^2 > D'_{ST}{}^2$) suggest that selective forces are not responsible for the observed disequilibria. Non-directional forces such as founder effects, population subdivision and parental sampling effects (Yeh and Morgan, 1987) represent possible causal factors. However, the consistency of the direction of the relationship between AK*2* and UGPU appears to suggest directional forces, *i.e.* epistatic selection. It should also be noted that, in partially-selfing species such as *A. excelsum*, (see Chapter 5; Ghazoul and McLeish, 2001), the expected or identity disequilibrium is non-zero (Hartl and Clark, 1989), and selfing itself tends to retard decay of disequilibrium (Falconer, 1989, Hartl and Clark, 1989).

For many applications, *e.g.* mating systems studies, the causes of observed disequilibria are of less interest than their magnitude. Close linkage, structural or otherwise, leads to underestimates of outcrossing rates (Brown *et al.*, 1985; Yeh and Morgan, 1987). In the present case, the consistency and magnitude of the AK*2*-UGPU correlation suggest that only one of this locus pair should be employed in mating system studies.

Neutrality

With the exception of PGM, the significant departures from expectations would appear to be best explained by three factors unrelated to selective non-neutrality. First, given the total number of estimates (60), three to four estimates significant at 5 per cent probability are expected due to chance alone. Second, three of the estimates in question pertain to the El Rodeo and E.J.N. populations, in which cases samples were dominated by one tree. Third, significant departure of *F* from expectations may also reflect recent population bottlenecks (Hartl and Clark, 1989). In the case of PGM, the pooled results suggested that the generally high expected heterozygosity maintained at the locus may be due to balancing selection. However, the absence of any tendency to heterozygote excess in progeny arrays of putatively heterozygous mothers suggests that such selection is not important at the sampled life-stage.

Applications

The loci LAP, PGD, PGM and UGPU appear to be unlinked, selectively neutral and codominantly inherited. As such, they are suitable for applications based on these assumptions. Although the presence of double-banded, putatively heterozygous phenotypes suggests that AK2 is codominantly inherited, it appears to be unsuitable for mating systems applications, both because of strong linkage with UGPU and the interpretation difficulties noted above. The latter also imply that estimates of measures of genetic variation based on AK*2* could be biased downwards, *i.e.* because of possible masking of an additional allele. Although the foregoing analysis suggests that admixture of extraneous seed in progeny arrays, if occurring at all, is sporadic and therefore unlikely to cause large errors in estimates of mating system parameters, caution dictates that such estimates should nevertheless be regarded as tentative.

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Table 4-1. Locations and sampling details of Costa Rican *Anacardium excelsum* populations sampled in a study of inheritance, linkage and neutrality of six isozyme systems

population	Coordinates[1]	NMT[2]	NI[3]	$\bar{x}$, range[4]
El Cepo	85°07.5’, 10°28.5’	19	420	22.1, 8-20
El Ojoche	85°06.3’, 10°29.6’	8	128	16, 13-32
Las Congojas	85°06.6’, 10°28.5’	20	270	13.5, 3-20
River Santa Rosa	85°06.3’, 10°27.6’	10	158	15.8, 3-21
La Isla	85°06.9’, 10°23.7’	5	81	16.2, 3-21
Palmira	85°05.6’, 10°33’	4	72	18, 16-21
Paso Hondo	85°10.5’, 10°23.3’	4	50	12.5, 7-19
Bosque Duquesa	85°07’, 10°19.5’	4	74	16, 15-20
E.J.N.	85°08.5′, 10°20’	2	17	8.5, 1-16
El Rodeo	85°04.3’, 10°19’	3	21	7, 3-21
Marcela	85°06’, 10°18.2’	14	154	11, 2-20
Toronja	85°09.3’, 10°18.3’	12	117	9.75, 3-20

[1] At population centre; [2] Number of mother-trees sampled; [3] Number of individuals sampled; [4] Respectively: mean, range in number of individuals sampled tree^{-1}

Table 4-2. Populations included in analysis of estimated proportion of extraneous seed in putative progeny arrays of putatively heterozygous individuals of *Anacardium excelsum*

Population/ Locus	Number of putatively heterozygous trees included in study	Excluded trees[1]
El Cepo		
AK*2*	5	1554
PGD	8	1551, 1566
PGM	10	
UGPU	5	
El Ojoche		
PGM	3	
Las Congojas		
AK*2*	5	6, 17
LAP	4	
PGM	7	
UGPU	4	
Marcela		
LAP[2]	8	419
PGM	6	
UGPU	3	
R.S.R.		
AK	3	
PGD	4	
PGM	3	
Toronja		
PGD	6	

[1] i.e. because extreme segregation ratio suggests spurious heterozygous designation (see text)

Table 4-3. Segregation ratios in pooled progeny arrays of mother trees putatively heterozygous for five allozyme loci, *Anacardium excelsum*

Enzyme	Maternal genotype	Classes for G-test	Observed numbers per class	n_{total}	G_{adj}[1]
AK*2*	AB	AA+BB	259	435	15.9***
		AB	176		
LAP	AB	AA+BB	247	464	1.94 N.S.
		AB	217		
LAP	AC	AA+CC	22	47	0.19 N.S.
		AC	25		
LAP	AB	AC	6	12	0.00 N.S.
		BC	6		
PGD	AB	AA+BB	321	546	16.90***
		AB	225		
PGM	AB	AA+BB	335	671	0.001
		AB	334		
UGPU	AB	AA+BB	181	334	2.35 N.S.
		AB	153		

[1] G-statistic with Williams's adjustment (Sokal and Rohlf, 1995); N.S. indicates p>0.05, * indicates 0.05>p>0.01; ** indicates 0.01>p>0.001; *** indicates p<0.001.

Table 4-4. Segregation ratios and associated probabilities in progeny of mother trees of *Anacardium excelsum* putatively heterozygous for the AK*2* locus.

Population	Family	n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}	n_{total}	n_{exp}[1]	p[2]
Duquesa	706	5	4	9	5	14	7	0.42
El Cepo	1512	8	3	11	9	20	10	0.82
El Cepo	1521	3	2	5	7	12	6	0.77
El Cepo	1532	5	4	9	11	20	10	0.82
El Cepo	1550	5	4	9	11	20	10	0.82
El Cepo	1551	2	3	5	11	16	8	0.21
El Cepo	1554	15	4	19	0	19	9.5	**<0.00**
El Rodeo	6	5	3	8	10	18	9.0	0.81
La Isla	15	10	3	13	7	20	10	0.26
Las Congojas	4	6	6	12	8	20	10	0.50
Las Congojas	5	10	3	13	6	19	9.5	0.17
Las Congojas	6	1	9	10	2	12	6.0	0.04
Las Congojas	8	4	3	7	5	12	6	0.77
Las Congojas	16	6	7	13	7	20	10	0.26
Las Congojas	17	1	8	9	1	10	5	0.02
Las Congojas	20	8	2	10	10	20	10	1.00
E.J.N.	110	7	1	8	7	15	7.5	1.00
Marcela	416	10	2	12	4	16	8	0.08
Palmira	1	3	5	8	5	13	6.5	0.58
Palmira	4	6	10	16	5	21	10.5	0.03
Paso Hondo	2	4	3	7	6	13	6.5	1.00
Paso Hondo	3	4	1	5	2	7	3.5	0.45
R.S.R.	3	7	2	9	10	19	9.5	1.00
R.S.R.	7a	6	4	10	10	20	10	1.00
R.S.R.	13	8	4	12	8	20	10	0.50
Toronja	115	8	2	10	9	19	9.5	1.00

[1] *i.e.* expected number in either n_{AB} or $n_{AA}+n_{BB}$ under null hypothesis of 1:1 ratio; [2] two-tailed exact binomial cumulative probability of n_{AB} if $n_{AB}<n_{AA}+n_{BB}$, or of $n_{AA}+n_{BB}$ if $n_{AB}>n_{AA}+n_{BB}$; probability values in bold are significant under Dunn-Šidák sequential Bonferroni test

Table 4-5. Segregation ratios and associated probabilities in progeny of mother trees of *Anacardium excelsum* putatively heterozygous for the LAP locus.

Maternal genotype, population	Family	Number of progeny per genotypic class				n_{total}	n_{exp} per class[1]	p[2]
AB mothers		n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}			
Duquesa	706	2	3	5	13	18	9	0.10
El Cepo	1513	5	2	7	8	15	7.5	1.00
El Cepo	1535	6	1	7	3	10	5	0.34
El Cepo	1566	16	1	17	3	20	10	0.003
El Ojoche	10	2	2	4	6	10	5	0.75
El Ojoche	21	7	7	14	18	32	16	0.60
La Isla	3	2	7	9	11	20	10	0.82
La Isla	4	4	2	6	12	18	9	0.24
La Isla	15	4	3	7	9	16	8	0.80
Las Congojas	5	3	1	4	11	15	7.5	0.12
Las Congojas	9	3	5	8	10	18	9	0.81
Las Congojas	16	10	2	12	6	18	9	0.24
Las Congojas	25	2	2	4	6	10	5	0.75
Marcela	304	2	1	3	4	7	3.5	1.00
Marcela	357	9	1	10	7	17	8.5	0.63
Marcela	371	2	1	3	0	3	1.5	0.25
Marcela	409	3	5	8	9	17	8.5	1.00
Marcela	415	2	6	8	10	18	9	0.81
Marcela	416	6	4	10	8	18	9	0.81
Marcela	418	5	2	7	3	10	5	0.34
Marcela	419	13	1	14	1	15	7.5	**0.001**
Marcela	503	5	3	8	4	12	6	0.39
Palmira	1	2	1	3	13	16	8.0	0.02
Palmira	2	6	5	10	8	18	9	0.81
Palmira	3	5	7	12	3	15	7.5	0.04
Palmira	4	4	8	12	7	19	9.5	0.36
R.S.R.	0	6	5	11	6	17	8.5	0.33
R.S.R.	5	1	8	9	3	12	6	0.15
R.S.R.	13	4	5	9	11	20	10	0.82
'Toronja'	116	5	1	6	4	10	5	0.75
		n_{AC}	n_{BC}					
El Cepo	1554	1	1			2	1	1.00
Las Congojas	5	0	1			1	0.5	1.00
Palmira	3	1	1			2	1.0	1.00
La Isla	4	0	1			1	0.5	1.00
Marcela	357	3	0			3	1.5	0.25
El Ojoche	10	0	2			2	1	0.50
R.S.R.	5	1	0			1	0.5	1.00

[1] *i.e.* expected number in either of the genotypic classes being compared under null hypothesis of 1:1 ratio; [2] two-tailed exact binomial cumulative probability of less frequent class; probability values in bold are significant under Dunn-Šidák sequential Bonferroni test

Table 4-5. Segregation ratios and associated probabilities in progeny of mother trees of *Anacardium excelsum* putatively heterozygous for the LAP locus (continued)

Population, maternal genotypes	Family	Number of progeny per genotypic class				n_{total}	n_{exp} per class	*p*[1]
AC mothers		n_{AA}	n_{CC}	$n_{AA}+n_{CC}$	N_{AC}			
El Cepo	1550	2	2	4	6	10	5	0.75
El Cepo	1551	3	6	9	8	17	8.5	1.00
El Ojoche	12	3	2	5	7	12	6	0.77
El Ojoche	16	3	1	4	4	8	4	1.00
BC mothers		N_{AC}	N_{BC}					
La Isla	1	2	6	8	3	11	5.5	0.23

[1] *i.e.* expected number in either of the genotypic classes being compared under null hypothesis of 1:1 ratio; [2] two-tailed exact binomial cumulative probability of less frequent class.

Table 4-6. Segregation ratios and associated probabilities in progeny of mother trees of *Anacardium excelsum* putatively heterozygous for the PGD locus.

Population	Family	n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}	n_{total}	n_{exp}[1]	p[2]
Duquesa	700	8	1	9	11	20	10	0.82
Duquesa	706	2	4	6	8	14	7	0.79
Duquesa	700a	4	3	7	13	20	10	0.26
El Cepo	1511	18	1	19	1	20	10	**<0.00**
El Cepo	1512	8	4	12	8	20	10	0.50
El Cepo	1521	8	2	10	8	18	9	0.81
El Cepo	1535	4	5	9	11	20	10	0.82
El Cepo	1542	4	5	9	10	19	9.5	1.00
El Cepo	1550	5	5	10	10	20	10	1.00
El Cepo	1551	7	1	8	11	19	9.5	0.65
El Cepo	1554	14	1	15	5	20	10	0.04
El Cepo	1556	7	3	10	7	17	8.5	0.63
El Cepo	1566	17	1	18	2	20	10	**<0.00**
Ojoche	2923	9	1	10	4	14	7	0.18
La Isla	1	11	2	13	5	18	9	0.10
La Isla	3	5	4	9	12	21	10.5	0.66
Las Congojas	6	9	5	14	5	19	9.5	0.06
Marcela	371	1	1	2	1	3	1.5	1.00
Marcela	418	7	2	9	1	10	5	0.02
Palmira	1	4	7	11	5	16	8.0	0.21
Palmira	4	10	6	16	4	20	10	0.01
Paso Hondo	2	2	8	10	3	13	6.5	0.09
Paso Hondo	3	1	6	7	0	7	3.5	0.02
R.S.R.	0	8	1	9	7	16	8	0.80
R.S.R.	3	5	3	8	12	20	10	0.50
R.S.R.	5	4	3	7	7	14	7	1.00
R.S.R.	7a	9	4	13	7	20	10	0.26
Toronja	104	7	4	11	9	20	10	0.82
Toronja	107	3	6	9	6	15	7.5	0.61
Toronja	108	2	2	4	1	5	2.5	0.38
Toronja	115	5	1	6	13	19	9.5	0.17
Toronja	116	4	1	5	5	10	5	1.00
Toronja	115	5	1	6	13	19	9.5	0.17

[1] *i.e.* expected number in either of either n_{AB} or $n_{AA}+n_{BB}$ under null hypothesis of 1:1 ratio; [2] two-tailed exact binomial cumulative probability of n_{AB} if $n_{AB}<n_{AA}+n_{BB}$, or of $n_{AA}+n_{BB}$ if $n_{AB}>n_{AA}+n_{BB}$; probability values in bold are significant under Dunn-Šidák sequential Bonferroni test

Table 4-7. Segregation ratios and associated probabilities in progeny of mother trees of *Anacardium excelsum* putatively heterozygous for the PGM*1* locus.

Population	Family	n_{AA}	n_{BB}	$n_{\mathrm{AA}}+n_{\mathrm{BB}}$	n_{AB}	n_{total}	n_{exp}[1]	p[2]
Duquesa	700	5	4	9	11	20	10	0.82
Duquesa	700A	7	4	11	9	20	10	0.82
El Cepo	1511	4	6	10	10	20	10	1.00
El Cepo	1512	4	5	9	11	20	10	0.82
El Cepo	1521	4	5	9	11	20	10	0.82
El Cepo	1539	1	3	4	6	10	5	0.75
El Cepo	1549	8	8	16	4	20	10	0.01
El Cepo	1550	1	2	3	7	10	5	0.34
El Cepo	1553	5	7	12	8	20	10	0.50
El Cepo	1554	3	1	4	5	9	4.5	1.00
El Cepo	1556	6	3	9	9	18	9	1.00
El Cepo	1566	2	11	13	5	18	9	0.10
El Ojoche	16	2	4	6	9	15	7.5	0.61
El Ojoche	21	7	5	12	20	32	16	0.22
El Ojoche	23	3	5	8	6	14	7	0.79
La Isla	1	7	4	11	7	18	9	0.48
La Isla	3	4	3	7	14	21	10.5	0.19
La Isla	15	4	7	11	9	20	10	0.82
Las Congojas	4	2	1	3	7	10	5	0.34
Las Congojas	6	7	4	11	9	20	10	0.82
Las Congojas	9	6	4	10	10	20	10	1.00
Las Congojas	12	8	3	11	9	20	10	0.82
Las Congojas	17	4	4	8	2	10	5	0.11
Las Congojas	22	10	2	12	8	20	10	0.50
Las Congojas	24	6	1	7	3	10	5	0.34
MAG	110	2	3	5	11	16	8	0.21
Marcela	357	6	6	12	7	19	9.5	0.36
Marcela	415	6	2	8	11	19	9.5	0.65
Marcela	416	3	4	7	11	18	9	0.48
Marcela	418	6	1	7	3	10	5	0.34
Marcela	419	3	4	7	8	15	7.5	1.00
Marcela	503	1	4	5	7	12	6	0.77
Palmira	3	3	4	7	12	19	9.5	0.36
Paso Hondo	1	6	1	7	3	10	5.0	0.34
Paso Hondo	2	7	2	9	4	13	6.5	0.27
R.S.R.	0	2	6	8	9	17	8.5	1.00
R.S.R.	5	1	7	8	5	13	6.5	0.58
R.S.R.	7	3	2	5	15	18	9	0.04
Toronja	115	8	2	10	10	20	10	1.00
Toronja	116	3	1	4	9	13	6.5	0.27

[1] *i.e.* expected number in either of either n_{AB} or $n_{\mathrm{AA}}+n_{\mathrm{BB}}$ under null hypothesis of 1:1 ratio; [2] two-tailed exact binomial cumulative probability of n_{AB} if $n_{\mathrm{AB}}<n_{\mathrm{AA}}+n_{\mathrm{BB}}$, or of $n_{\mathrm{AA}}+n_{\mathrm{BB}}$ if $n_{\mathrm{AB}}>n_{\mathrm{AA}}+n_{\mathrm{BB}}$.

Table 4-8. Segregation ratios and associated probabilities of putatively heterozygous mother trees of *Anacardium excelsum*, UTP-glucose-1-phosphate uridylyltransferase

Population	Family	n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}	n_{total}	n_{exp}^1	p^2
Duquesa	706	5	1	6	7	13	6.5	1.00
El Cepo	1512	6	2	8	12	20	10	0.50
El Cepo	1535	8	2	10	10	20	10	1.00
El Cepo	1541	9	2	11	9	20	10	0.82
El Cepo	1550	4	4	8	12	20	10	0.50
El Cepo	1557	6	2	8	6	14	7	0.79
El Ojoche	10	2	3	5	8	13	6.5	0.58
El Ojoche	12	2	3	5	5	10	5	1.00
Las Congojas	1	4	2	6	10	16	8	0.45
Las Congojas	4	4	3	7	13	20	10	0.26
Las Congojas	8	2	1	3	9	12	6	0.15
Las Congojas	20	10	3	13	7	20	10	0.26
Marcela	415	11	1	12	5	17	8.5	0.14
Marcela	416	7	2	9	9	18	9	1.00
Marcela	418	5	1	6	4	10	5	0.75
Palmira	1	5	4	9	6	15	7.5	0.61
Palmira	2	7	8	15	5	20	10	0.04
Palmira	4	8	6	14	7	21	10.5	0.19
R.S.R.	4	6	6	12	6	18	9	0.24
R.S.R.	5	11	1	12	2	14	7	0.01
R.S.R.	9	1	1	2	1	3	1.5	1.00

[1] *i.e.* expected number in either of either n_{AB} or $n_{AA}+n_{BB}$ under null hypothesis of 1:1 ratio; [2] two-tailed exact binomial cumulative probability of n_{AB} if $n_{AB}<n_{AA}+n_{BB}$, or of $n_{AA}+n_{BB}$ if $n_{AB}>n_{AA}+n_{BB}$. [1] *i.e.* of either n_{AB} or $n_{AA}+n_{BB}$;

Table 4-9. Estimates of proportions of extraneous seed in collections of putatively heterozygous mother trees of *Anacardium excelsum.*

Population/ Locus	N_{XX}:N_{XY} (arrays)[1]	XX:XY (arrays)[2]	XX:XY(pop)[3]	p_e[4]
El Cepo				
AK*2*	39:45	0.46:0.54	0.78: 0.22	-ve
PGD	83:70	0.54:0.46	0.76:0.24	0.15
PGM	89:76	0.54:0.46	0.67-0.33	0.23
UGPU	45:49	0.48:0.52	0.80-0.20	-ve
El Ojoche				
PGM	26:35	0.43:0.57	0.47-0.53	>1.0
Las Congojas				
AK*2*	55:36	0.60:0.40	0.77-0.23	0.37
LAP	28:33	0.46:0.54	0.82:0.18	-ve
PGM	62:48	0.56:0.44	0.58-0.42	0.75
UGPU	29:39	0.43:0.57	0.77:0.23	-ve
Marcela				
LAP^{2}	57:45	0.56:0.44	0.83:0.17	0.18
PGM	46:47	0.49:0.51	0.56:0.44	-ve
UGPU	27:18	0.60:0.40	0.84:0.16	0.29
R.S.R.				
AK	31:28	0.52:0.48	0.69:0.31	0.11
PGD	37:33	0.53:0.47	0.76:0.24	0.12
PGM	21:29	0.42:0.58	0.70:0.30	-ve
Toronja				
PGD	41:47	0.46:0.54	0.56:0.44	-ve

[1]Numbers of homozygotes and heterozygotes in offspring of putative heterozygotes; [2]Ratio of homozygotes to heterozygotes in collections from putative heterozygotes; [3]Ratio of homozygotes to heterozygotes in population as a whole; [4]estimated proportion of extraneous seed.

Table 4-10. Results of simulation of effect on estimation of mating system parameters of presence of extraneous seed in progeny arrays for combinations of two initial outcrossing rate scenarios ($t_m \approx 1.0$, $t_m \approx 0.7$), three allele frequency scenarios and four seed admixture scenarios

Case[1]	Allele Frequencies	Settings (N, e)[2]	Parameter estimates (s.d.) before admixture	Parameter estimates (s.d.) after admixture		Number of aborted runs[3]
				F	t_m	
1.1	.85, .10, .05	1: 20, 10	F=-.038 (.149) t_m=1.112 (.038)	1: -0.97(0.039)	1: 1.31 (0.072)	0
	.48, .52	2: 20, 2		2:0.36 (0.12)	2: 1.14 (0.020)	0
	.44, .56	3: 2, 10		3: -0.14 (0.054)	3: 1.11 (0.017)	0
		4: 2, 2		4: -0.06 (0.024)	4: 1.11 (0.005)	0
1.2	.77, .15, .08	1: 20, 10	F=-.169 (.136), t_m=1.027 (.043)	1: -0.96 (0.071)	1: 1.21 (0.041)	1
	.49, .51	2: 20, 2		2: -0.52 (0.106)	2: 1.02 (0.015)	2
	.83, .17	3: 2, 10		3: -0.26 (0.05)	3: .99 (0.009)	0
		4: 2, 2		4: -0.195 (0.03)	4: .99 (0.007)	0
1.3	.81, .12, .07	1: 20, 10	F=.11 (.366), t_m=1.003 (.031)	1:-0.98 (0.025)	1: 1.14 (0.037)	0
	.82, .18	2: 20, 2		2: -0.99 (0.000)	2: 1.01 (0.033)	1
	.87, .13	3: 2, 10		3: 0.09 (0.054)	3: 1.01 (0.007)	0
		4: 2, 2		4: 0.11 (0.006)	4: 1.005 (0.002)	1
2.1	.79, .14, .07	1: 20, 10	F=-.028 (.145), t_m= .759 (.039)	1: -0.98 (0.02)	1: 1.11 (0.081)	3
	.58, .42	2: 20, 2		2: -0.42 (0.131)	2: .81 (0.049)	0
	.48, .52	3: 2, 10		3: -0.11 (0.045)	3: .78 (0.018)	2
		4: 2, 2		4: 0.058 (0.018)	4: .76 (0.007)	0
2.2	.84, .11, .05	1: 20, 10	F=-.021 (.17), t_m=.716 (.038)	1: -0.99 (0.00)	1: 1.08 (0.039)	4
	.44, .56	2: 20, 2		2: -0.51 (0.083)	2: .826 (0.019)	2
	.87, .13	3: 2, 10		3: -0.11 (0.05)	3: 73 (0.02)	1
		4: 2, 2		4: -0.07 (0.046)	4: .72 (0.01)	0
2.3	.83,.14,. 03	1: 20, 10	F=.176 (.248), t_m=.75 (.054)	1: -0.98 (0.076)	1: 1.0 (0.023)	0
	.86,.14	2: 20, 2		2: -0.96 (0.058)	2: 0.78 (0.060)	0
	.82,.18	3: 2, 10		3: 0.105 (0.027)	3: 0.79 (0.017)	0
		4: 2, 2		4: 0.119 (0.053)	4: .74 (0.014)	0

[1] Case 1.n refers to outcrossing rate setting of $t_m \approx 1.0$, n defines allele frequency settings (see column 3), case 2.n refers to $t_m \approx 0.7$; [2] N=number of trees affected by admixture, e=number of admixed seed per affected tree, accordingly, designations 1,2,3 and 4 refer to generalized and intense seed admixture, generalized but light, sporadic but intense, sporadic and light, respectively; [3] i.e, due to presence of 3 homozygotic types in ≥1 array

Table 4-11. Significant estimates of Burrows's composite linkage disequilibrium coefficient (Δ_{ij}), correlation coefficient (r) and significance (p) of associated chi-squared test in populations of *Anacardium excelsum* in Guanacaste province, Costa Rica.

Loci and alleles[1]	**Population**	Δ_{ij}	r	p	n[2]	**+N:-N**[3]
AK2-A *v.* LAP-A	Toronja:	-.0564	-.2514	.0165	91	6/6
AK2-A *v.* UGPU2-A	Duquesa	.0252	.2627	.0258	73	10/0[4]
	Cepo	.0350	.1526	.0061	319	
	Santa Rosa	.0439	.1952	.0168	148	
AK2-A *v.*PGD-A	La Isla	0.0736	0.2746	0.0280	64	9/3
	Paso Hondo	-0.1104	0.3462	0.0102	55	
LAP-A *v.*PGM*1*-A	Duquesa	-0.0532	-0.27	0.0220	72	8/4

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[1] All alleles except the most frequent were pooled to one synthetic allele; [2] number of individual genotypes observed; [3] *i.e.* numbers of positive and negative correlations, irrespective of significance, over all 12 populations; [4] total is < total number of populations (12) because p=1 (>0.9999) in remaining populations, *i.e.* disequilibrium≈0;

Table 4-12. Two by three classification of *Anacardium excelsum* progeny by PGD putative genotype and presence of putative AK*1*-A allele, with result of chi-square test of association.

	Number of individuals per progeny class		
	AK*1*-A present	**AK*1*-A absent**	**totals**
PGD putative genotypes			
AA	917	41	958
AB	108	171	279
BB	9	117	126
totals	1034	329	1363
Chi², df, p:	741.55, d.f.=2, p=<0.0001		

Table 4-13. Estimates of Ohta's multiple population linkage disequilibrium coefficients for 12 populations of *Anacardium excelsum* in northwestern Costa Rica.

Loci	D_{IT}^2	D_{IS}^2	$D'_{IS}{}^2$	D_{ST}^2	$D'_{ST}{}^2$
AK2/LAP	0.16675	0.00156	0.15416	0.16216	0.01259
AK2/PGD	0.13579	0.00844	0.12970	0.12196	0.00609
AK2/PGM	0.12596	0.00386	0.11996	0.12666	0.00599
AK2/UGPU	0.12176	0.00177	0.11533	0.11955	0.00644
LAP/PGD	0.14757	0.00106	0.14351	0.14080	0.00406
LAP/PGM	0.12940	0.00114	0.12655	0.12781	0.00285
LAP/UGPU	0.13974	0.00034	0.13529	0.13852	0.00445
PGD/PGM	0.09803	0.00108	0.09750	0.09385	0.00053
PGD/UGPU	0.06787	0.00034	0.06611	0.06795	0.00176
PGM/UGPU	0.08043	0.00149	0.07894	0.08241	0.00149
Overall	**0.11035**	**0.00146**	**0.10619**	**0.10802**	**0.00416**

Table 4-14. Results of Ewens-Watterson test of selective neutrality for 12 populations of *Anacardium excelsum* in northwestern Costa Rica.

Locus	Parameter	Population[1]					
		D	C	O	R	I	Co
AK2	**n^2**	146	632	246	42	128	520
	F_{obs}^3	0.82	0.64	0.98	0.50	0.51	0.59
	95% CL[4]	0.50-.99	0.51-1.0	0.50-.99	0.50-.95	0.50-.98	0.50-1.0
LAP	**n^2**	146	718	218	42	148	508
	F_{obs}^3	0.65	0.80	0.50	0.95	0.51	0.76
	95% CL[4]	0.50-.99	0.51-1.0	0.50-.99	0.50-.95	0.50-.99	0.51-1.0
PGD	**n^2**	148	788	254	42	162	532
	F_{obs}^3	0.56	0.68	0.87	0.91	0.67	0.84
	95% CL[4]	0.50-.99	0.50-1.0	0.50-.99	0.50-.95	0.50-.99	0.51-1.0
PGM	**n^2**	146	582	248	42	162	408
	F_{obs}^3	0.60	0.58	0.52	0.66	0.51	0.56
	95% CL[4]	0.50-.99	0.50-.99	0.50-.99	0.50-.95	0.50-.99	0.50-1.0
UGPU	**n^2**	146	822	242	M[5]	160	532
	F_{obs}^3	0.88	0.74	0.77		0.98	0.75
	95% CL[4]	0.50-.99	0.50-1.0	0.51-.99		0.50-.99	0.50-1.0

[1]Population abbreviations: D=Bosque Duquesa, C=El Cepo; O=El Ojoche; R=El Rodeo; I=La Isla; Co=Las Congojas; E=E.J.N.; M=Marcela; P=Palmira; PH=Paso Hondo; S=R.S.R.; T=Toronja; [2]number of alleles in sample; [3]observed *F*; [4]95 per cent confidence limits of sampling distribution; [5]M=monomorphic

Table 4-14. Results of Ewens-Watterson test of selective neutrality for 12 populations of *Anacardium excelsum* in northwestern Costa Rica (continued).

Locus	**Parameter**	**Population**[1]						
		E	**M**	**P**	**PH**	**S**	**T**	**All**
AK*2*	**n**[2]	32	302	148	114	310	228	1766
	F$_{obs}$[3]	0.60	0.88	0.65	0.62	0.69	0.76	0.62
	95% CL[4]	0.50-.94	0.50-.99	0.50-.99	0.50-.98	0.50-.99	0.50-.99	0.5-0.99
LAP	**n**[2]	34	302	142	108	270	188	1780
	F$_{obs}$[3]	0.84	0.53	0.50	0.76	0.61	0.53	0.64
	95% CL[4]	0.50-.94	0.50-.99	0.50-.99	0.50-.98	0.50-.99	0.50-.99	0.50-.99
PGD	**n**[2]	34	306	150	114	312	226	1896
	F$_{obs}$[3]	0.94	0.83	0.57	0.60	0.69	0.54	0.73
	95% CL[4]	0.50-.94	0.50-.99	0.50-.99	0.50-.98	0.50-.99	0.50-.99	0.50-.99
PGM	**n**[2]	34	306	144	104	286	232	1698
	F$_{obs}$[3]	0.50	0.52	0.71	0.60	0.70	0.54	0.50
	95% CL[4]	0.50-.94	0.50-.99	0.50-.99	0.50-.98	0.50-.99	0.50-.99	0.50-.99
UGPU*2*	**n**[2]	M[5]	292	152	116	306	234	1912
	F$_{obs}$[3]		0.81	0.54	0.98	0.62	0.93	0.78
	95% CL[4]		0.50-.99	0.50-.99	0.50-.98	0.50-.99	0.50-.99	0.51-.99

[1] Population abbreviations: D=Bosque Duquesa, C=El Cepo; O=El Ojoche; R=El Rodeo; I=La Isla; Co=Las Congojas; E=E.J.N.; M=Marcela; P=Palmira; PH=Paso Hondo; S=R.S.R.; T=Toronja; [2] number of alleles in sample; [3] observed *F*; [4] 95 per cent confidence limits of sampling distribution; [5] M=monomorphic

Figure 4-1. Adenylate kinase zymogram of *Anacardium excelsum* (3 families), showing putative loci (marked '1' and '2'). 'C' indicates *Pinus resinosa* control.

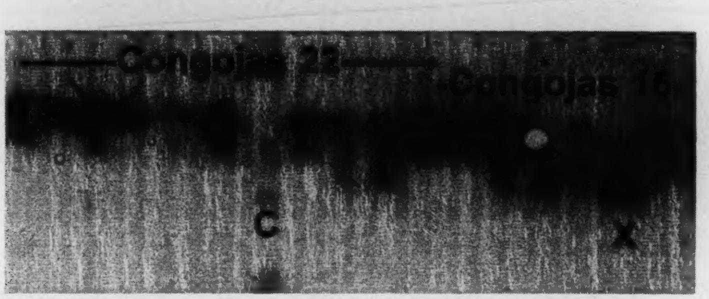

Figure 4-2. Adenylate kinase zymogram of *Anacardium excelsum* (2 families). 'X' indicates lane with most common AK*2* allele absent and intense staining cathodal of most common AK*1* band (see text). 'C' indicates *Pinus resinosa* control.

Figure 4-3. Leucine aminopeptidase zymogram of *Anacardium excelsum*, showing putative genotypes AA (marked '1'), BB (2), AB (3), CC (4), AC (5) in four arrays, and *Pinus resinosa* controls (C).

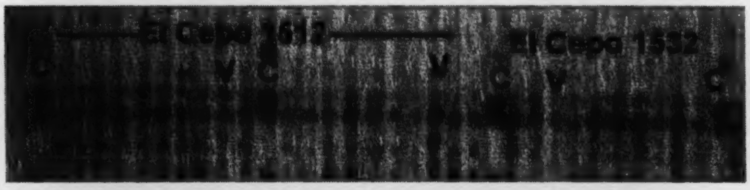

Figure 4-4. Malate dehydrogenase zymogram of *Anacardium excelsum*, showing variant phenotypes (marked V) in two progeny arrays and *Pinus resinosa* controls (C).

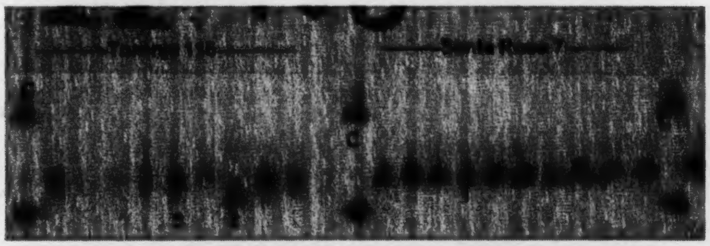

Figure 4-5. Phosphogluconate dehydrogenase zymogram of *Anacardium excelsum*, showing putative genotypes AA (marked '1'), BB (2), AB (3) in two progeny arrays, and *Pinus resinosa* controls (C).

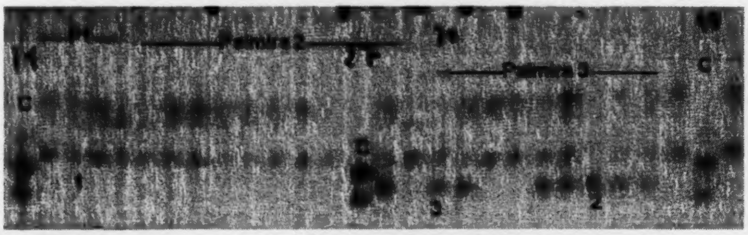

Figure 4-6. Phosphoglucomutase zymogram of *A. excelsum*, showing: putative genotypes in three arrays (AA (marked '1'), BB (2), AB (3)); faint artifactual banding in PGM-*B* position (BB genotypes marked '2' and those adjacent (right)); *Pinus resinosa* controls (C)

Paso Hondo 1

Figure 4-7. UTP-glucose-1-phosphate uridylyltransferase zymogram of *Anacardium excelsum*, showing putative genotypes AA (marked '1'), BB (2), AB (3) in two progeny arrays, and *Pinus resinosa* controls (C).

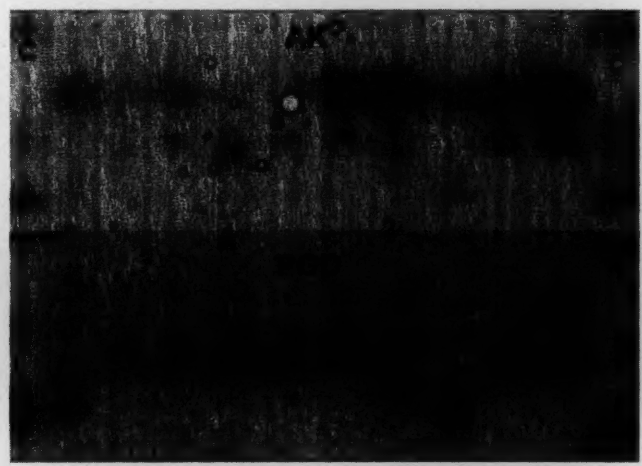

Figure 4-8. Adenylate kinase (AK) (above) and phosphogluconate dehydrogenase (PGD) (below) zymograms for *A. excelsum* (family Marcela 418), showing apparent linkage (putative PGD genotypes: 1=AA, 2=AB, 3=BB. C indicates *Pinus resinosa* control.

Chapter 5

THE EFFECTS OF FOREST FRAGMENTATION ON GENETICS AND REPRODUCTION OF THE TREE *ANACARDIUM EXCELSUM* (BERTERO & BALBIS) SKEELS (ANACARDIACEAE) IN NORTHWESTERN COSTA RICA

INTRODUCTION

Tropical forests were destroyed at a rate of around 15.2 million ha year^{-1} in the decade 1990-2000 (FAO, 2001). However, deforestation is often not complete; rather, in many cases, one or more tracts within the formerly continuous tree cover remain forested, and are converted by deforestation into fragments set in an unforested matrix (Riitters *et al.*; 2000, Schelhas, 1996). If biologically viable, such fragments may mitigate some negative consequences of deforestation. It follows that the biological implications, including genetic aspects, of the conversion of forest tracts to forest fragments are of considerable relevance to the management and conservation of forests and biodiversity.

The possible effects of forest fragmentation on genetic diversity are complex and interacting. The most immediate of these effects occurs at fragmentation, which, for species formerly present in deforested matrices, leads to reduction in population size. Depending on their size and allele frequencies, reduced populations may not contain all alleles formerly present, *i.e.* they may show founder effects (Meffe and Carroll, 1994; Yeh 2000). Continued low population size is expected to lead to further loss of variation due to random genetic drift (Hartl and Clark, 1989), a process which may be exacerbated by changed post-fragmentation environment. The latter has the potential to cause additional reductions in population size and higher variation in fertility (*e.g.* Aldrich and Hamrick, 1998; Kelly *et al.*, 2000), thereby reducing the ratio N_e/N (effective to census population sizes) (Nunney, 1993; Falconer, 1989). The effects of fragmentation on inter- and intra-population gene flow may exacerbate or mitigate such responses. For example, disturbance-mediated declines in density of tree populations may, due to decline in inter-tree pollinator movements (Karron *et al.*, 1995; Ghazoul *et al.* 1998), lead to increased geitonogamous selfing, which may also be caused by disturbance-mediated changes in pollinator assemblages (Aizen and Feinsinger, 1994). Increased selfing may have an immediate negative impact on fitness, *e.g.* by causing inbreeding depression (Gigord *et al.* 1998), and also increases susceptibility to drift by further reducing N_e (Yeh 2000). Maintenance of prefragmentation levels of gene flow may, with time, restore variation lost in founder events and may also prevent cumulative drift. Although the proportion of immigrant seed and pollen in a given fragment x may be lower than when the fragment was a tract in continuous forest, sources of post-fragmentation immigrant seed and pollen may tend to be located further away from x than prefragmentation sources. When genetic distance correlates with physical distance, such migrants may be more effective in preventing genetic drift, because of their greater genetic divergence (Mills and Allendorf, 1996). Furthermore, as suggested above, fragmentation and concomitant disturbance may reduce the number of pollination events involving 'home' pollen, implying higher migration rate for a given amount of incoming pollen.

Clearly, the effect of fragmentation on genetic diversity is not easy to predict, particularly given the relatively limited empirical information available on responses of tree species to fragmentation (see Chapter Two). In the present document, the effects of forest fragmentation on population genetics, flowering, seed size and seedling growth rates of *Anacardium excelsum* are reported. *A. excelsum* is a large, evergreen tree native from Honduras to Ecuador and the Guyanas (Hartshorn and Gentry, 1991). The small, andromonoecious (Mitchell and Mori, 1987) flowers, which Ghazoul and McLeish (2001) reported as partially self-incompatible, are borne on large (up to 50cm length) panicles. In the study zone, trees flower annually between January and April (Cornelius, J.P., personal observation); large individuals may produce several hundred panicles. The most common floral visitors tend to be small native bees (*Trigona* spp.) (Ghazoul and McLeish, 2001). After fertilization, the pedicel expands to a fleshy, twisted hypocarp, which bears a hard drupe at its distal end (Hartshorn and Gentry, 1991; Mitchell and Mori, 1987), which typically matures by early to mid-April (Cornelius, J.P., personal observation). The sweet hypocarps are consumed by bats and howler monkeys (Glander, 1979; Hartshorn and Gentry, 1991; Mitchell and Mori, 1987), which discard or drop the toxic drupes. Many also drop under gravity to the forest floor beneath fruiting trees. Seeds will germinate immediately on moist ground, *e.g.* on stream sides. Frequently, however, seeds lie in the dry litter until the wet season sets in, *i.e.* typically no earlier than mid-May. *A. excelsum* seeds do not form a long-term soil seed bank (Cornelius, J.P., personal observation).

In seasonally-dry zones, as in the Pacific watershed of Central America, *A. excelsum* is generally found in moist sites less subject to seasonal drought, *e.g.* riparian forests. Its conservation ecology in such sites is of interest for two reasons. Firstly, as a locally common species of naturally clumped distribution, information about the population genetics of *A. excelsum* may generate insights of relevance to other taxa with similar life histories and similarly patchy or fragmented distributions. Secondly, gallery forest appears to represent a large proportion of the remaining closed forest of this largely deforested zone (Chapter Three); as a dominant and common species of such forest, the 'genetic health' of *A. excelsum* populations is linked to the conservation status of these habitats.

METHODS

Study zone and fragments

The study zone is an area of approximately 350 km^2 located between 10°33' and 10°18' N, 85°02'° and 85°12'W in the *cantones* of Cañas and Bagaces, Guanacaste Province, northwestern Costa Rica (Chapter Three; Figure 5-1). Around 95% of the mean annual rainfall of 1693.4 mm (s=459.4) falls between May and November (San Luis, Cañas meteorological station, 1921-1978, MIRENEM, 1988). Altitude varies from 20-200m a.s.l.; mean annual temperature at 95m a.s.l. is 27.5°C (Jiménez *et al.*, 1987). The dry season is characterized by strong (up to 90km hr^{-1}) northerly winds (Coen, 1983) and temperatures up to 37°C. The main land uses in the study zone are industrial agriculture (sugar-cane and rice) and beef cattle ranching. The former predominates on the mollisols, alfisols, vertisols and alluvial inceptisols found south and immediately north of the Interamerican Highway (Figure 5.1), which cuts through the study zone, whilst ranching predominates on the shallower soils to the north of the highway (Chapter Three). The deforestation of the study zone appears to have occurred mostly during the last 80 years, as a consequence of three main factors: the replacement by exotic pasture species of the semi-open woodland ('sitios') formerly used for grazing, the conversion of closed forest to grassland, and the conversion of woodland to sugar-cane and rice production (Chapter Three). Within the zone, 25 forest remnants containing *A. excelsum* were located using maps, aerial photographs and field exploration. They include non-linear (non-riparian) and linear (mostly riparian) fragments, with wide variation in population size, disturbance, isolation and matrix type (Table 5-1).

Genetic variation, gene flow and mating systems

Field and laboratory procedures

All individual *A. excelsum* trees >10cm dbh in each fragment were mapped and their dbh measured. In April 1999, seeds were collected beneath panicle-bearing branches of individual trees in 12 fragments (Table 5-2). In larger populations, seed trees were randomly selected, whilst in smaller populations collections were made from all fruiting trees. The collections were made from the forest floor directly beneath the selected trees, avoiding areas beneath overlapping crowns of neighbouring trees. The seeds were germinated in July and August of the same year, and starch gel electrophoresis was carried out on enzyme extracts prepared from seedling leaf tissue. Gels were stained for five codominantly inherited (see Chapter Four) enzyme systems: AK (adenylate kinase, E.C. 2.7.4.3), LAP (leucine aminopeptidase, E.C.3.4.11.1), PGD (phosphogluconate dehydrogenase, E.C. 1.1.1.43), PGM (phosphoglucomutase, E.C. 5.3.1.9) and UGPU (UTP-glucose-1-phosphate uridylyltransferase, 2.7.7.9) (laboratory protocols are detailed in Appendix One). These were selected from a wider group of enzymes based on resolution, polymorphism and consistency with expected quaternary structure (Chapter Four).

Population genetic analysis

Genetic parameters were estimated for maternal and progeny generations. As maternal material was unavailable, maternal genotypes were inferred using the most-likely-parent method (Brown and Allard, 1970), as programmed in Ritland's MLTR program (DOS version 1.1) (Ritland, 1996). For both generations, allele frequencies and allelic richness (A) (mean number of alleles per locus) were calculated. As all the sampled loci were polymorphic, A in this case is equivalent to AP (mean number of alleles per polymorphic locus) (Berg and Hamrick, 1997). In order to permit comparison of allelic richness between generations, estimates of progeny allelic richness were then adjusted to the respective maternal sample size using Hurlbert's (1971) rarefaction method, implicitly assuming that population sizes would remain constant over generations. For example, if 10 mother-trees were sampled, progeny allelic richness was rarefied to the expected value for 10 sampled progeny. Rarefaction was carried out using

Brzustowski's (undated) on-line calculator. The null hypothesis of no differences between parental and progeny A was tested using the signed rank test (Sokal and Rohlf, 1995). Nei's expected heterozygosity ($H_e = 1 - \sum_i^n p^2{}_i$, where p_i=frequency of allele i) (Weir, 1996) was also estimated and averaged over all assayed loci. The fixation index F_{is} ($1\text{-}H_o/H_e$, where H_o=observed heterozygosity), was calculated as a measure of heterozygote deficit or excess with regard to Hardy-Weinberg equilibrium (HWE) expectations. The null hypothesis of HWE within each population was tested using G-tests. In the case of the triallelic locus LAP, all alleles but the most common were pooled to one synthetic class for both the significance testing (to maximize the number of observations per expected genotypic class) and calculation of F_{is} (for consistency between the measure of disequilibrium and its test statistics). However, due to the low population numbers, in many cases the number of observations in the least common class was still ≤ 5, usually considered as the threshold for uncritical application of the G-test (Sokal and Rohlf, 1995).

Nine of the 12 sampled fragments fall into two geographic groups, termed here the 'Corobicí group' and the 'Taboga group' (Table 5-1, Figure 5-1). The latter is composed of a set of discrete linear and non-linear fragments set in the sugar-cane matrix of the Taboga estate. It includes the large 'Marcela' fragment and a number of smaller remnants. The Corobicí group fragments are located on the River Corobicí and its tributaries; the matrix is mostly pastureland (Chapter Three). For the populations as a whole, and by these groups, homogeneity tests were carried out to test the null hypothesis of no population differentiation. As for the tests of Hardy-Weinberg equilibrium, and for the same reasons, the less common LAP alleles were pooled to one synthetic allele. Wright's F-statistics (F_{is}, correlation between uniting gametes relative to the subpopulations; F_{it}, correlation between uniting gametes relative to the population as a whole; F_{st}, correlation between two gametes drawn randomly within subpopulations relative to that of two gametes drawn randomly from the population as a whole) were estimated as:

$$F_{IS} = \frac{H_S - H_I}{H_S},$$

$$F_{IT} = \frac{H_T - H_I}{H_T},$$

$$F_{ST} = \frac{H_T - H_S}{H_T},$$

where H_I = observed average heterozygosity of individuals within subpopulations; H_S=expected average heterozygosity; H_T= total expected heterozygosity (gene diversity) for population as a whole (pooled subpopulations) (Yeh, 2000).

Average historical gene-flow was estimated using the F_{st} method (*i.e.* $mN=0.25(1-F_{st})/F_{st}$), where N=effective population size and m = average migration rate) (Yeh, 2000). Pairwise estimates of Nei's unbiased genetic distance were also calculated and UPGMA dendrograms (Weir, 1996) based on these were generated. Significance of the relationship between pairwise genetic distance and pairwise geographical distance between fragments was tested using the Mantel test (Sokal and Rohlf, 1995) as programmed in Mantel Version 2.0 (Liedloff 1999).

Except where indicated, the above analyses were carried out using the software package POPGENE (Yeh and Boyle, 1997).

Current gene flow

The Paso Hondo population, which was completely sampled (sample size = 4 = population size) is monomorphic for UGPU and also lacks LAP-C (see results). In this case, gene flow is estimable as:

$$m = \frac{q_t}{\overline{q}},$$

where m=proportion of immigrant alleles, q_t=allele frequency in the progeny generation, $\overline{q}$ = mean allele frequency in the source population (Hamrick and Nason, 2000). Paso Hondo is a highly isolated population (Figure 5-1), with no single obvious source population. Therefore, $\overline{q}$ was set to the average maternal allele frequency over all populations ($\overline{q}$ =0.15, 0.07 for UGPU, LAP-C, respectively).

Gene flow from the outlying tree Bosque Duquesa 706 (Figure 5-2) to the other three sampled trees of this population was also examined (tree 706 is heterozygous for UGPU, whilst the remaining sampled trees are homozygous AA). In this case, gene flow estimates from tree 706 represent maxima, as the allele could be present in the remaining five unsampled trees of the population or in immigrant pollen.

Estimation of mating system parameters

The multilocus outcrossing rate (t_m), average single-locus outcrossing rate (t_s) correlations of outcrossing rate (r_t) and outcrossed paternity (r_p) were estimated by fragment using Ritland's MLTR programme (DOS Version 1.1) (Ritland, 1996), which employs Ritland and Jain's (1981) mixed mating system model. Within MLTR, the likelihood equations were solved using the EM method, and standard errors ($s_{\bar{x}}$) computed with 1000 bootstraps. Estimates of t less than $1-2s_{\bar{x}}$ were considered to depart significantly from full outcrossing (Liengsiri *et al.*, 1998); analogous criteria were used for r_p, r_t, *i.e.* estimates greater than $2s_{\bar{x}}$ were considered to differ significantly from zero. For all MLTR analyses, all available parameters were simultaneously estimated. The AK*2* locus was omitted, because it appears to be linked with UGPU (Chapter 4); linkage tends to lead to underestimation of outcrossing rate (Brown *et al.*, 1985, Yeh and Morgan, 1987). Two progeny arrays with three LAP homozygotes (see Chapter Four), indicative of more than one contributing maternal parent, were excluded from the data sets. Estimates of individual-tree outcrossing rates were not made because of the relatively small numbers of progeny per maternal family (Table 5-2). Tree density was selected as a possible explanatory variable for variation in outcrossing rate, because plant density effects on mating systems and pollinator behaviour have been documented previously (Ghazoul *et al.*, 1998; Karron *et al.*, 1995; Murawski and Hamrick, 1991; Murawski and Hamrick, 1992). In a regression analysis of outcrossing rates on density, the following distance-weighted neighbourhood density index (*NDI*) was used:

$$NDI = \frac{\sum_{i=1}^{n}\left(t_{25} + 0.5t_{50} + 0.25t_{100}\right)_i}{n}$$

n = number of sampled trees, $t_{25/50/100}$ = number of mature *A. excelsum* trees within 0-25m, 25-50m, 50-100m of each sample tree (determined from mapping data).

The expected equilibrium inbreeding coefficient was calculated using the formula:

$$\hat{F}_e = \frac{1-t_m}{1+t_m}$$

(Fyfe and Bailey, 1951; Liengsiri *et al.*, 1998). The relative values of $\hat{F}_e$ and observed F_{is} are of interest as they may help determine whether observed t_m values have shown recent changes, *e.g.* due to fragmentation. Therefore, the relationship between the two variables was examined graphically and by correlation analysis.

Flowering

Field observations of both flowering and fruit production during the 1997 seed collection season suggested that both these fertility parameters varied between fragments. In particular, many trees in fragments located in dry and/or exposed situations (*e.g.* Avispa, Duquesa Arriba, Isla, Salitral, Toronja) were not observed to produce flowers or fruits. This phenomenon is of relevance to the genetic impact of fragmentation, because the mean and variance of fertility are related to population viability both directly (mean), and through their relationship with effective population size (variance) (Nunney, 1993; Falconer, 1989; Wright, 1938). Accordingly, in March and April 1998, the number of panicles visible with binoculars from the ground on trees in each of 24 fragments (Table 5-2) were counted. The large panicles of *A. excelsum* persist throughout the flowering and fruiting season. In smaller populations, counts were made on all trees. In large populations, 10 trees were selected randomly (sample sizes are listed in Table 5-2). Observations were not made on trees with dbh<60cm as, for purposes of characterizing within-population variation in fertility, variation due to age variation was considered of less interest than fragmentation-induced environmental variation. The mean number of panicles tree^{-1} fragment^{-1} was calculated as an index of fertility. As an index of flowering variability, Shannon's equitability index (*J*) (Begon *et al.*, 1998) was used:

$$J = \frac{-\sum_{i=1}^{S} P_i \ln P_i}{\ln S},$$

where P_i = proportion of total number of panicles contributed by the i^{th} sample tree; S= number of sample trees. Equitability, which varies from zero to one, is maximal when all individuals produce the same number of panicles. Zero values cannot be used in the above formula. This characteristic is of no concern with the commonest ecological application of J, *i.e.* in species equitability. In the present context, however, omission of non-flowering trees would give a misleading impression of fertility variation. For example, if two trees each produce 100 panicles, whereas eight produce none, then J=1, although gamete production would actually be strongly dominated by the two individuals. Because of this, zero values were eliminated by increasing by one the panicle count of each tree.

As there was significant heterogeneity of variance for both the log-transformed and the untransformed mean flowering counts, the null hypothesis of no differences in number of panicles tree^{-1} fragment^{-1} was tested using the Kruskal-Wallis test for independent samples (Sokal and Rohlf, 1995). Differences between fragments were highly significant (see results). In order to attempt to elucidate the causal factors involved, the following linear regression model was used:

$$\hat{I} = \alpha + \beta type + \lambda dbh,$$

where $\hat{I}$ = expected mean number of panicles for specified values of variables *type* and *dbh*; *type* = 1 for fragments in well-sheltered locations on watercourses, and 0 otherwise (type 1 fragments are indicated in Table 5-14), *dbh* = mean diameter at breast height of sampled trees.

In the case of fertility equitability, interfragment differences cannot be tested because there is only one datum per fragment. Consequently, a regression approach was used with the same model as for the flowering counts. Additionally, a simple linear regression of equitability on flowering count itself was also tested. Except where otherwise stated, statistical analyses of these and all other variables (see below) were done with SAS (1999).

Seed weight and growth rate

There are a number of mechanisms by which fragmentation might negatively affect *in situ* or *ex situ* growth rate of seedlings. Firstly, changed levels of conditions and resources, *e.g.* due to edge effects, may affect growth or competitive success of regeneration (*in situ*). Secondly, the same factors might affect seed development, which in turn may correlate with seedling growth rate, *ex situ* or *in situ*. Thirdly, increased homozygosity due to drift or inbreeding may result in inbreeding depression (Charlesworth and Charlesworth, 1987; Gigord *et al.*, 1998; Husband and Schemske, 1996; Hedrick and Kalinowski, 2000), again affecting both *in situ* and *ex situ* growth rate.

In order to test for such effects, two common garden experiments were carried out. In April and May 1997, seeds were collected from individual trees in the fragments El Cepo, Canateca, Marcela, Quebrada Reventado (South), Central/South Q. Salitral (bulked), Q. Duquesa/Bosque Duquesa (bulked) and Toronja. Random samples of 55 seed were taken for each by drawing equal numbers of seeds from the individual family lots available for each fragment. The seeds were weighed individually to the nearest centigram. In order to test for fragment effects on seed weight, the data were analyzed by analysis of variance under the following model:

$$Y_{ij} = \mu + \alpha_j + e_{ij},$$

where Y_{ij} = weight of the i^{th} seed of the j^{th} fragment; μ=overall mean; α_j = effect of the j^{th} fragment; e_{ij} = residual associated with the i^{th} seed of the j^{th} fragment. Least square means were estimated by fragment and pairwise testing carried out using Bonferroni-adjusted *t*-tests.

On 15th July 1997, the seeds were directly sown in a nursery bed in Turrialba, Costa Rica under 50 per cent shade. Plant spacing was 17cm by 17cm and experimental design was randomized complete blocks (five blocks, 11 plants per plot). The experiment was hand-watered as necessary. Shade netting was removed on 18th August 1997. As no border rows was used, and because hand-watering tends to favour central rows, blocks were laid out longitudinally along the 1m wide nursery bed. Germination date for each seed was noted. Total height was measured to the nearest cm on 17th November, *i.e.* four months after sowing. In order to test for fragment effects on four-month height and germination day, analysis of variance under the following mixed model was carried out:

$$Y_{ijk} = \mu + \alpha_j + \beta_k + e_{ijk},$$

where Y_{ijk} = height of the i^{th} seedling of the j^{th} fragment in the k^{th} block; μ=overall mean; α_j = fixed effect of the j^{th} fragment; β_k = random effect of the k^{th} block, e_{ijk} = residual associated with the i^{th} seedling of the j^{th} fragment in the k^{th} block. This reduced model was derived from an initial fuller model including block-by-fragment interaction; the latter effect was dropped because it was insignificant. Least square means were estimated by fragment and pairwise testing carried out using Bonferroni-adjusted *t*-tests. As there were significant fragment effects on seed weight (see results), an analysis of covariance was also carried out using the same model with the addition of the seed weight covariate. Additionally, germination date was used as covariate in a similar analysis of effects on height growth. Five abnormal seedlings (characterized by shrivelled first leaves, diseased cotyledons or failure to shed testa and release cotyledons and first true leaves) (one in each of the fragments Cepo, Duquesa, Reventado, Salitral and Toronja) were excluded from the analysis of height. Although it is possible that these problems might be genetic in origin, fungal attack or other deterioration after dispersal seem more likely explanations.

The presence of fragment effects on germination percentage was tested using ANOVA under the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij},$$

where Y_{ij} = germination percentage of seeds of the ith fragment in the jth block, transformed by arcsin; μ=overall mean; α_i = fixed effect of the i^{th} fragment; β_j = random effect of the j^{th} block, e_{ij} = residual associated with the estimate of germination percentage of the i^{th} fragment in the j^{th} block.

A second common-garden test was established on 12 August 1999 in University of Alberta greenhouse facilities. In order to test for inbreeding effects on growth rate, the same fragments assayed for the mating systems study were included (Table 5-2) (with the exception of Paso Hondo, for which number of available maternal seedlots was less than the adopted minimum of four per fragment). A randomized complete block design with 10 blocks and single-tree family plots was used. Seedlots of each family were sown in germination trays and transplanted to 12.7cm diameter plastic pots filled with MetroMix™ (Monsanto) after emergence of the hypocotyl but before shedding of the testa. Excess germinants were also transplanted and held in reserve in the same greenhouse. The experimental pots were placed contiguously, *i.e.* without additional space between pots, giving 12.7cm (equivalent to pot diameter) between plants. In 169 of the experimental germinants, it became apparent after shedding of the testa that one or more cotyledons were damaged or destroyed, apparently due to deterioration during storage or germination. Sixty-seven of these plants died during the three weeks after establishment, and were replaced by randomly selected germinants from the 'reserve'. The remaining 102 damaged plants survived but did not recover normal growth rate. They were therefore excluded from the subsequent analysis of the experiment. In addition, 126 plants had not shed testas by day 23 and were showing no signs of continuing development. Testas of these plants were cut off with nail scissors. Thirty of these plants had damaged or infected cotyledons, and are included in the total of 102 mentioned above, *i.e.* they were excluded from the analysis. The resulting final representation of families by block is detailed in Table 5-3. Total height (81 days, 167 days), diameter at 2cm above the root collar (167 days) and, as a measure of seed size, testa length (mm) after shedding were measured. Least squared fragment means were estimated and significance of fragment differences tested by analysis of variance under the following mixed linear model:

$$Y_{ij(k)l} = \mu + \alpha_{j(k)} + \beta_k + \lambda_l + \delta_{ij(k)kl},$$

where $Y_{ij(k)l}$ value of the response variable on the ith seedling of the jth family of the kth fragment in the lth block; μ = experimental mean, $\alpha_{j(k)}$ =random effect of the jth family in the kth fragment; β_k =fixed effect of the kth fragment; λ_l =random effect of the lth block; $\delta_{ij(k)kl}$ = residual error associated with the ijlth observation. This reduced model was derived from an initial fuller model including block-by-fragment interaction; the latter effect was dropped because it was insignificant for all variables. Similarly to the 1997 study, analysis of covariance was also carried out with date of testa shedding or removal and seed coat length as covariables.

The analyses were carried out using SAS under the GLM procedure and the Analyst tool (SAS, 1999). Expected mean squares are included in Table 5-22 and 5-24.

Albino seedlings were noted in three of the sown families (Cepo 1505, 1541 and Ojoche 21). These were replaced with normal seedlings. Additional seed of these families was sown on 2nd December 1999 in order to estimate the proportion of albino seedlings in each case.

RESULTS

Genetic variation and structure

Maternal generation

All alleles of all loci were present in sampled maternal genotypes of the El Cepo, El Ojoche, and River Santa Rosa populations (Table 5-4). Allele LAP-C was absent from the sampled maternal genotypes of populations Bosque Duquesa, Las Congojas, Marcela, Palmira and Paso Hondo. UGPU-A was fixed in samples from La Isla, Paso Hondo y Toronja. Gene diversity (H_e) varied from 0.30 (Paso Hondo) to 0.39 (La Isla), and showed no relationship with population size (Figure 5-3). F_{is} values tended strongly to be negative; significant heterozygote excess was noted in LAP (El Ojoche, Marcela, Palmira), PGD (Cepo), PGM (Cepo, Congojas) (Table 5-4).

The homogeneity tests (Table 5-5) indicated that the populations as a whole are heterogenous at all studied loci. The overall F_{st} estimate of 0.18 (Table 5-6) suggests moderate to large genetic differentiation (Yeh 2000). Genetic distances (Table 5-7) appeared to be related to geographic distances (Figure 5-4), although the one-tailed *p*-value of 0.09 for the Mantel test (Z=142.2, G=1.04) was suggestive rather than conclusive. The pairwise genetic distances between the Paso Hondo and other populations were much higher than the others (Table 5-7). On removing this population, the relationship between genetic and geographical distances became strongly significant (Z=78.19, G=2.18, r=0.39, one-tailed p=0.015). Homogeneity tests (Table 5-5) and F_{st} (Table 5-6) estimates by group suggest similar, low-to-moderate genetic differentiation within the Taboga and Corobicí groups. UPGMA dendrograms for the 10 populations and two groups are illustrated in Figure 5-5 to 5-7.

Progeny generation

All alleles of all loci were present in progeny genotypes of all populations except Bosque Duquesa (LAP-C), El Rodeo (LAP-C, UGPU-B) and EJN (LAP-C, UGPU-B) (Table 5-8). When rarefied to the maternal sample size, progeny allelic richness was significantly lower than maternal allelic richness (signed rank test: sum of positive ranks = 51.5, sum of negative ranks = 3.5, $p<0.02$). Gene diversity (H_e) varied from 0.20 (El Rodeo) to 0.41 (Palmira) and was not linearly related to log population size (Figure 5-8). F_{is} values tended strongly to be positive; significant homozygote excess was noted in at least one locus of all populations, and all loci showed significant homozygote excess in at least one population. Mean values ranged from 0.06 (El Ojoche) to 0.27 (Paso Hondo) (Table 5-8)

The homogeneity tests indicate that the populations as a whole are heterogenous at all studied loci (Table 5-9). The overall F_{st} estimate of 0.18 (Table 5-10) suggests moderate to large genetic differentiation (Yeh 2000).

Genetic distances (Table 5-11) appeared to be related to geographic distances (Figure 5-9), although the Mantel test statistic (Figure 5-9) was not significant. Genetic distances between the Paso Hondo and other populations were unusually high, but the relationship between genetic and log geographic distance was not made stronger or more significant by removing this population. Homogeneity tests (Table 5-9) and F_{st} (Table 5-10) estimates by group suggest rather greater genetic differentiation within the Corobicí group than the Taboga group. UPGMA dendrograms for all 12 sampled populations and two groups are illustrated in Figures 5-10 to 5-12.

Gene flow

For maternal and progeny data, mean estimates of gene flow based on the F_{st} method were around 1 individual generation^{-1} for the populations as a whole, but from 2-4 individuals generation^{-1} within the Corobicí and Taboga groups (Tables 5-6, 5-10).

The mean estimated migration rate into the Paso Hondo population was 0.18 (Table 5-12). There was zero estimated gene flow from Bosque Duquesa Tree 706 to the other three sampled trees, *i.e.* UGPU-B was absent from their progeny (Table 5-8).

Mating system parameters

Estimates of t_m (Table 5-13) ranged from 0.266 (Palmira) to 0.744 (El Ojoche). All estimates departed significantly from 100 per cent outcrossing. Estimates of t_s ranged from 0.258 (Palmira) to 0.726 (Marcela), but were generally higher than corresponding multilocus estimates. All estimates departed significantly from 100 per cent outcrossing, except for La Isla and Paso Hondo. Correlation of outcrossing rate (r_t) were low, ranging from 0.086 (Santa Rosa) to 0.230 (Palmira), but significant in six cases. Correlation of outcrossed paternity (r_p) ranged from 0.418 (Bosque Duquesa) to 0.980 (Toronja), and departed significantly from zero in all cases. $\hat{F}_e$ ranged from 0.15 (Ojoche) to 0.58 (Palmira) and appeared to be positively linearly correlated with observed F_{is} (Figure 5-13). In seven cases $\hat{F}_e$ was greater than F_{is}; the latter was marginally greater in the three fragments where this was not the case.

Multilocus outcrossing rate was significantly and strongly positively related to population mean neighbourhood density (t_m=0.4182+0.0071*ndi*, F=6.83, p=0.0309, R^2=0.461) (Figure 5-15).

Flowering and seed weight

Fragment mean flowering indices varied from 1.9 tree^{-1} to 211 tree^{-1} (Table 5-14). There were highly significant differences between populations in tree flowering indices (Kruskal-Wallis test, chi-square = 83.4, df=23, p<0.0001). Both mean dbh and fragment type had positive and significant effects on mean flowering index (Table 5-15), i.e. fragments of type 1 (sheltered, on watercourses) had higher production of flowers.

Population equitability (J) values varied from 0.17 to 0.85 (Table 5-14). In several cases, one tree produced >50% of the total panicle count. J was significantly positively affected by *type*, but not by dbh mean or standard deviation (Table 5-16). J was significantly correlated with mean flowering index (Figure 5-14).

There was a highly significant effect of origin (fragment) on seed weight (Table 5-17), mean weights ranging from 1.72g (Duquesa) to 2.59g (Cepo) (Table 5-18). Mean seed weights in the El Cepo and Canateca fragments were significantly higher than those of the other fragments. There were no significant differences between the Toronja, Salitral and Duquesa fragments, and these had significantly lower weights than all the other fragments apart from Quebrada Reventado (Table 5-18).

Common garden experiments

1997 experiment

The overall germination rate was 45 percent. There was a highly significant fragment effect on arcsin germination percentage (Table 5-19). Germination percentage varied from 22 per cent (Salitral) to 78 percent (Marcela) (Table 5-20).

The mean height at four months was 27.0cm (s=7.31cm). ANOVA revealed highly significant fragment effects on four month height (Table 5-19); least square means varied from 22.1cm (Toronja) to 31.5cm (Marcela) (Table 5-20).

Mean time to germination was 15.5 days (s=2.22), and germination day was significantly affected by fragment of origin (Table 5-19); least square mean germination day varied from 14.1 (Reventado) to 16.0 (Duquesa, Salitral) (Table 5-20). Seed weight and germination day were respectively significant and highly significant as covariates, but a strongly significant fragment effect on growth rate remained (Table 5-21). Least squares height means from the covariance analysis are included in Table 5-20.

1999 experiment

Analysis of variance revealed a significant effect of fragment on height at both measurement ages but not on diameter or testa length (Table 5-22). There were no significant pairwise differences. The relationships of least square means of the three growth variables (Table 5-22) with outcrossing rate were not significant (Figures 5-16 to 5-18).

In the analysis of covariance, both covariates were highly significant for all three variables (Table 5-24). Significance of fragment effects persisted (Table 5-25). Again, there were no significant relationships with outcrossing rate (Figures 5-19 to 5-21) or significant pairwise differences.

The numbers of albinos produced by families 1505, 1541 and Ojoche 21 are detailed in Table 5-26. The segregation ratio for Ojoche 21 (31:13) was not significantly different from 3:1 ($G=0.41$, $G_{crit,\ p=0.05)} = 3.84$). Albino seedlings from this seedlot are pictured in Figure 5-22.

DISCUSSION

Comparison of levels of within-population gene diversity shown by *A. excelsum* with those of published meta-analyses is not straightforward, as the latter (*e.g.* 0.13±0.011 for native tropical woody taxa, Loveless, 1992) are inclusive of monomorphic loci. However, as Loveless reported percentage of loci polymorphic (*P*) of 39.0 per cent for the same group, this implies mean gene diversity of polymorphic loci for this group of (0.13/0.39) = 0.33, i.e. closely similar to the estimates (means of 0.34, 0.33 in maternal and progeny generations) reported here. Similarly, the estimates for *A. excelsum* are comparable to other estimates based on polymorphic allozyme loci of neotropical trees, *e.g.* for *Enterolobium cyclocarpum* ($H_e=0.365$, five loci, Rocha and Lobo, 1996), *Pithecellobium elegans* ($H_e=0.31$, recalculated for 6 polymorphic loci, Hall *et al.*, 1994a), *Pentaclethra macroloba* ($H_e=0.21$, three loci of adult trees, Hall *et al.*, 1994b), *Carapa guianensis* ($H_e=0.31$, 6 polymorphic loci, Hall *et al.* 1994c). There is, at least, no indication from the data - i.e. genetic variation at polymorphic loci - that *A. excelsum* shows any general tendency to low levels of within-population gene diversity.

Nevertheless, the results of the present study indicate clearly that forest fragmentation threatens this *status quo*, essentially due to the action of several mutually reinforcing processes, *i.e.* founder effects, declining and variable fertility, higher inbreeding and lower growth rates. These are considered below. Subsequently, the potential of gene flow and selection to mitigate these factors is discussed.

Founder effects

The reduced allelic richness of the completely sampled Paso Hondo population (maternal generation) provides the clearest evidence of founder effects: two of the alleles present in the group of populations as a whole are absent in the four mother-trees which make up the population. Although records suggest that this population has been isolated since at least the first part of the last century (see Chapter Three), the large size of the trees (mean dbh 151.3cm) suggests that all four trees derive from prefragmentation mating events, *i.e.* that cumulative drift is unlikely to be responsible. Founder effects also seem to be responsible for the notable genetic divergence of the population, as quantified in the genetic distance matrices.

In the case of the other populations (excepting Palmira, which was completely sampled, and from which an allele (LAP-C) has also been lost) progeny data are more informative, as they sample more completely the populations in question. Three populations (Bosque Duquesa, El Rodeo, and E.J.N.), exhibit progeny allelic richness lower than the maximum of A=2.2. In the case of Bosque Duquesa, it is possible that the absence of LAP-C from the progeny arrays reflects loss of this allele from the population, in which >50% of the adult trees were sampled. However, the possibility that one or more unsampled trees heterozygous or homozygous for LAP-C failed to contribute to the sampled pollen pool cannot be discounted. Sampling intensity was lower in E.J.N. and El Rodeo, suggesting that sampling effects, rather than founder effects, may be responsible in these cases.

Flowering and seed weight

The strong effect of fragment type on flowering fertility suggests resource limitation of flowering in some fragments. The three fragments with particularly low levels (COSTASEM, Las Avispas, Quebrada Salitral) are all located in the largely treeless (Chapter 3) and flat sugar-cane and rice matrix of the southern part of the study zone. These fragments lack protection from exposure; Las Avispas also appears unusually dry for *A. excelsum* habitat, presumably due to the drainage-works in the surrounding cane-fields.

In many cases, reduced flowering may imply lower seed production, independently of whether there are subsequent additional (pollinator or resource) limitations on the latter. Although no formal study of seed production was made, the expectation of reduced seed production is born out by experience in seed collection in the southern part of the study zone. For example, in the 1997 collection season, mean numbers of seed per tree collected in the Avispa, Duquesa Arriba, Isla and Salitral Central fragments ranged from 2.0-4.4. By contrast, in the large riparian fragments north of the Interamerican highway, numbers of seed collected were in general an order of magnitude greater (in practice, a collection ceiling of 30-50 seeds tree^{-1} was set. Ghazoul and McLeish's (2001) findings on seed production in nine of these fragments are consistent with these observations. They found notably reduced mature seed production in the Avispa and Paso Hondo ('Tres Espavels') fragments.

The results suggest that the same factors that give rise to declining flowering are also likely to increase variance in flowering fertility, *i.e.* deteriorating conditions and resource levels, under which only a few trees are able to produce normal numbers of seed and flowers, leading to dominance of reproduction by one or a few trees. A similar 'secondary bottleneck' in response to fragmentation has been reported by Aldrich and Hamrick (1998) in *Symphonia globulifera.* Both low mean fertility and such bottlenecks tend both to exacerbate initial founder effects and predispose populations to ongoing drift. Low mean fertility would tend to cause continuing population decline, or at least would inhibit population growth, whilst high variance in fertility tends to be the most important cause of N_e being less than N (Falconer,1989).

Fragment effects on seed weight and size appear to parallel the effects on flowering; with the single exception of the Marcela fragment (for seed weight), degraded, exposed and/or dry fragments have the lowest seed weights (Duquesa, Salitral, Toronja) and testa lengths (Bosque Duquesa, Toronja, Palmira). Clearly, this suggests that the same resource limitations that affect flowering may also have independent effects on seed development.

Inbreeding

The observed relationship between neighbourhood density and outcrossing implies that pollinator movement is limited by distance. This is indirectly supported by the high correlations of paternity of outcrossed progeny (suggesting that a few neighbouring trees may be responsible), by the current (zero) gene flow estimate for the Bosque Duquesa population, and by Ghazoul and McLeish's (2001) data on insect visitation to flowers in five of these fragments. When all five fragments in common to both studies are considered, there is a positive but insignificant correlation between t_m estimates reported here and the number of insect visits per unit time reported by Ghazoul and McLeish (r=0.43, p=0.47, df=3). However, the latter authors found that the Paso Hondo ('Tres Espavels') fragment had a markedly different flower visitor assemblage to all the other fragments, with 17 per cent of visits by *Trigona* bees, against >50 percent in the other fragments. When this fragment is disregarded, there is a significant, near perfect correlation between insect visitation frequency and t_m (r=0.96, p=0.04, df=2).

The relationship between outcrossing rate and neighbourhood density has implications for the effect of forest fragmentation on genetics of *A. excelsum*, as reduction of neighbourhood density in *A. excelsum* is an expected consequence of fragmentation in three related scenarios. First, in riparian fragments, as the width of the gallery forest 'buffer' is reduced, the forest is effectively redimensioned from plane to line, with a corresponding reduction in neighbourhood density. This effect can be particularly strong when one river bank is completely deforested, as is common in the study zone. Secondly, conditions in the disturbed fragments may favour other species at the expense of *A. excelsum*, *e.g.* initially, successionally earlier species, and, subsequently, species better suited to the drier, hotter conditions. Finally, as demonstrated here, post-fragmentation conditions may be inimicable to flowering, seed production, seed development and seedling growth (see below) and, therefore, for all four reasons, to recruitment. In effect, there is a conversion to marginal habitat, which is expected to offer fewer suitable microsites and higher probability of competitive exclusion (Yeh and Layton, 1979) and will therefore lead to lower stocking per unit area.

The segregation ratio for albinism in tree Ojoche 21 is strongly suggestive of selfing, or near selfing, of an individual heterozygous for the locus controlling this trait. The sporadic occurrence of albinism in other families may be caused either by outcrossing events between heterozygous individuals or lower degrees of self-fertilization of heterozygous individuals. The presence of this lethal allele in two of the populations demonstrates that inbreeding, particularly selfing, in *A. excelsum* can have an adverse effect on fitness. Ghazoul and McLeish (2001) also found that selfed seed of the species had higher abortion rates. It follows that the higher selfing rates likely to be caused by fragmentation-mediated density reduction may directly to reduced viability fitness.

The observed positive relationship between F_{is} and $\hat{F}_e$ suggests that increased selfing is already affecting genetic variation in these fragments. At the same time, the tendency for $\hat{F}_e$ to be higher than F_{is} indicates that equilibrium values have not yet been reached, and therefore that the most serious effects of increased inbreeding have yet to be manifested.

It is worth noting that at least some of the outcrossing estimates are surprisingly low, and are suggestive of complete self-compatibility, as reported by Freitas and Paxton (1996) for cashew (*A. occidentale*), rather than the partial self-incompatibility reported by Ghazoul and McLeish (2001). Roubik (1995) also reported self-compatibility in *A. giganteum*.

Seedling growth rate

The analyses suggest that the observed variation in growth rate between seedlings from different fragments is partly due to variation in seed weight and size, implying that the probable causal factors implicated in the latter (*i.e.* resource limitation) also affect seedling growth rate. This factor may be a sufficient explanation for the similarity in the results of the two trials, *i.e.* the poor performance in both of the Toronja and Duquesa sources. The superiority in both trials of plants from the Marcela fragment cannot be explained in these terms. It is possible that lower inbreeding depression might be one cause of the superiority of the Marcela source, which is the largest of the non-riparian populations and has relatively high t_m and relatively low r_p (suggesting a low tendency to biparental inbreeding). However, as the relationship between outcrossing rate and growth in the second trial is, at strongest (Figure 5-18), no more than suggestive, this explanation can be no more than tentative. The lack of any clear relationship between growth rate and outcrossing rate may imply that inbreeding depression is expressed predominantly at earlier life stages, *e.g.* immediately after fertilization, as observations by Ghazoul and McLeish (2001) suggest.

In summary, *A. excelsum* populations in the study fragments show, variously, evidence of founder effects, excess homozygosity in progeny, lower (rarefied) allelic richness in progeny than adults, 'secondary bottlenecks' related to fragment conditions and higher selfing rates due to lower density. The possible roles of gene flow and selection in mitigating these problems are now considered.

Gene flow

Gene flow between populations tends to result in increased intra-population genetic variation. It has the potential to restore genetic variation lost in founder effects, to counteract cumulative drift, and to reduce selfing (because seed produced from immigrant pollen is necessarily outcrossed). One such effect can be seen directly in the present study, *i.e.* in the 'reintroduction' of LAP-C and both UGPU-B and LAP-C to, respectively, Palmira and Paso Hondo (these alleles are absent in maternal generations, but present at low frequency in the progeny, due to gene flow). However, without substantial increases in population sizes, these alleles are unlikely to be present in actual progeny generations (consistent with this, they are not reflected in rarefied allelic richness).

Theoretically, the low-to-moderate degree of subpopulation-within-group differentiation suggests that gene flow at this level has been substantial, *i.e.* 3-4 migrants generation^{-1}. However, there are grounds for caution in concluding from these data that gene flow will be insufficient to mitigate the negative impacts mentioned above. Firstly, gene flow between the populations as a whole appears to have been notably less (as evinced by the high overall F_{st} values). The estimate of F_{st}=0.18 is higher than average G_{st} values for comparable species, *e.g.* long-lived woody species in general (0.084±0.008, Hamrick *et al.*, 1992), and, within this group, mixed breeding system, animal-pollinated species (0.122±0.038), gravity-attached seed dispersal (0.099±0.024), and much higher than Loveless's (1992) mean value for biotically dispersed tropical species (0.050±0.008). The plots of genetic *v.* geographic distance reveal obvious relationships, disrupted by individual exceptional pairwise combinations, *i.e.* those due to the Paso Hondo population in the case of the maternal generation, and, in the case of the progeny, the Ojoche-Marcela pairwise observation, which has geographic and genetic distances of 21km and 0.008, respectively (Figure 5-5). Correlations between genetic and geographic distance imply isolation-by-distance (IBD) (Yeh, 2000). This may reflect both that, as expected, pollinators should forage no further afield than necessary to meet their energy requirements, and inability of a relatively weak-flying pollinator (*i.e. Trigona* spp.) to traverse larger distances. Either way, increasing distance between *A. excelsum* fragments might tend to promote shifts to foraging on alternative species rather than energy-expensive moves between widely separated fragments. Low levels of flowering in some fragments, as observed here, would also be expected to reduce probability of visitation by non-resident pollinators. Ghazoul and McLeish's (2001) finding of greatly reduced *Trigona* visitation in the isolated Paso Hondo fragment is consistent with this expectation. The presence of IBD suggests also that diaspore vectors are subject to similar constraints. Field studies of bat foraging in the study zone support this, e.g. Heithaus *et al.* (1975) found that although individuals of *Artibeus* and other taxa ranged relatively widely along the River Corobicí, none were recaptured in sample points 4km northwest, on the River Tenorito. Individuals carrying *A. excelsum* hypocarps with drupes still attached would be still rarer.

Secondly, even within the two groups, the UPGMA grouping is consistent with geographical expectations, suggesting presence of IBD also at this smaller geographic scale. The fragments Marcela and Duquesa group separately from the Toronja fragment, from which they are separated by a range of low hills (Las Lomas). The Cepo and Santa Rosa populations, which were connected by continuous riparian forest until the construction of the dam on the River Santa Rosa, group separately from the two non-linear fragments Ojoche and Congojas.

Thirdly, findings on current gene flow suggest that immigration rate may be idiosyncratic to particular fragments. The low-flowering Bosque Duquesa fragment shows no evidence of inward gene flow. The Paso Hondo fragment, by contrast, appears to be subject to substantial current gene flow; the migration rate estimate of 0.18 implies that 36% of pollen is of immigrant origin. However, even in this case, mN would still be relatively small, even if $N_e=N$.

Selection

F_{is} estimates in maternal generations, which tend to be negative, are in marked contrast to those for the progeny generations, which tend to show homozygote excess. It is possible that this may reflect either heterozygote advantage or selection against deleterious recessives. Both the latter and, in the case of asymmetrical overdominance, the former (Young *et al.*, 1996), may result in purging of genetic load. Such purging, although in itself tending to increase homozygosity, would tend to mitigate immediate effects on fitness of increased inbreeding. However, studies suggest that purging does not act consistently in natural populations, possibly because slightly deleterious alleles may principally be responsible for genetic load (Byers and Waller, 1999). Furthermore, purging would not prevent loss of currently neutral variation, which in changed environmental conditions may become adaptive.

As a whole, the results of this study suggest a species rather ill-equipped to deal with the threat posed by forest fragmentation. This is well exemplified by the case of the Bosque Duquesa fragment, which shows loss of variation due to founder effects, combined with at least five other factors expected to lead to continuing decline in census or effective population sizes (reduced flowering, low seed weight, slower seedling growth rate, high variance in fertility, low outcrossing), whilst there is little or no mitigating immigration. Although, as suggested by current levels of gene diversity, the impact of forest fragmentation on genetic variation of *A. excelsum* appears to be largely incipient rather than yet realized, it is not clear that either gene flow or selection will be able to obviate the likely adverse consequences.

A. excelsum shows fewest signs of fragmentation effects under the least disturbed conditions, and it seems probable that in large, relatively undisturbed riparian fragments the species will continue to persist indefinitely. However, the risk posed by disturbance of such habitat, whether caused by fragmentation or other factors such as grazing or damming (as in the River Santa Rosa fragment), remains clear.

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Table 5-1. Description of fragments sampled in a study of genetic effects of forest fragmentation on *Anacardium excelsum* in northwestern Costa Rica.

Fragment	coordinates[2]	N[3]	isolation[4]	disturbance[5]	matrix[6]	ndi[7], linearity[8]
Corobicí Group[1]						
El Cepo	85°07.5', 10°28.5'	500+	L	L	P	24.2, 2.9
El Ojoche	85°06.3', 10°29.6'	23	H (5)	M	P	17.0, 1.87
Las Congojas	85°06.6', 10°28.5'	50	M (10)	M	P	34.5, 1.92
Oldemar	85°06.7', 10°26.4'	15	M	H	P	n.d., riparian strip
Quebrada Lajero	85° 06.6', 10°29'	54	M (30)	H	P	n.d., 3.1
River Santa Rosa	85°06.3', 10°27.6'	19	H (5)	[illegible]	A/P	7.57, 15.0
Taboga Group[1]						
Bosque Duquesa	85°07', 10°19.5'	9	M (6)	L	P, S	5.0, 2.28
COSTASEM	85°09', 10°18.8'	10	M (4)	H	S, A	5.5, 5.25
E.J.N.	85°08.5', 10°20'	12	H (0)	L	P, A	22.4, 2.96
El Rodeo	85°04.3', 10°19'	6	H (n.d.)	H	P, S	7,2, 10.7
Hcda. Los Rios	85°05', 10°20'	15	H(0)	H	P	10.5, 9.44
La Gotera	85°04.2', 10°19.9'	109	L	L	P	2.4, 3.7
Las Avispas	85°08.2', 10°17.5'	27	H(0)	H	S	25.7, 5.22
Marcela	85°06', 10°18.2'	285	80 (M)	L	S	33.7, 2.96
Quebrada Duquesa	85°06.5', 10°20.1	10	H(1)	M	P, S	4.14, 4.7

[1]See text (methods); [2]At population centre; [3]Population size; [4]L=low, M=medium, H=high. Qualitative scale based on estimated number of trees of other fragments within 500m of population centre (in parentheses); where this data was unavailable, classifications were made based on field observations and knowledge of study zone; [5]L=low, M=medium; H=high. Qualitative scale based on field observations of degree of human intervention of each fragment; [6]P=pastureland, S=sugar-cane, A=other agriculture; [7]neighbourhood density index (see text); [8]*i.e.* population linearity : l/w, where l = length of a line drawn between the two most distant points of the population and w = maximum width of the population measured perpendicular to line l).

Table 5-1. Description of populations sampled in a study of genetic effects of forest fragmentation on *Anacardium excelsum* in northwestern Costa Rica (continued)

fragment[1]	coordinates[2]	N[3]	isolation[4]	disturbance[5]	matrix[6]	ndi[7], linearity[8]
Taboga group[1]						
R. Reventado (N)	85°05’, 10°19.2’	33	M (17)	H	P	n.d., 5.0
R. Reventado (S)	85°06.0’, 10°18.6’	57	L (100)	M	S	n.d., 2.33
Sitio Cascante	85°08.6’, 10°19’	15	H(10)	H	S	5.9, 3.25
Toronja	85°09.3’, 10°18.3’	34	H (0)	H	S	44.6, 1.06
Other fragments						
Canateca	85°09.6’, 10°31.7’	500+	L	L	P	n.d., riparian strip
Hcda. Tenorio	85°06’, 10°33.2’	500+	L	L	P	n.d., riparian strip
La Isla	85°06.9’, 10°23.7’	18	H (0)	M-H	S	10.6, 4.3
Palmira	85°05.6’, 10°33’	4	L (500)	H	P	4.25, 2.5
Paso Hondo	85°10.5’, 10°23.3’	4	H (0)	H	S	1.0, 14.75
Q. Salitral (Libertad)	85°06.5’, 10°22.9	22	M	H	S	n.d., riparian strip

[1]See text (methods); [2]At population centre; [3]Population size; [4]L=low, M=medium, H=high. Qualitative scale based on estimated number of trees of other fragments within 500m of population centre (in parentheses); where this data was unavailable, classifications were made based on field observations and knowledge of study zone; [5]L=low, M=medium; H=high. Qualitative scale based on field observations of degree of human intervention of each fragment; [6]P=pastureland, S=sugar-cane, A=other agriculture; [7]neighbourhood density index (see text); [8]*i.e.* population linearity : l/w, where l = length of a line drawn between the two most distant points of the population and w = maximum width of the population measured perpendicular to line l).

Table 5-2. Sampling of populations of in a study of effects of forest fragmentation on genetic and reproduction of *Anacardium excelsum* in northwestern Costa Rica.

fragment	genetic diversity[1]		mating system parameters		flowering study
	NF[2]	NI[3]	NF	NI	NI
Corobicí group					
El Cepo	19	420	19	420	10
El Ojoche	8	128	5	88	8
Las Congojas	20	270	17	259	7
Oldemar	0	0	0	0	11
Quebrada Lajero	0	0	0	0	7
River Santa Rosa	10	158	10	158	10
Taboga group					
Bosque La Duquesa	4	74	4	74	5
COSTASEM	0	0	0	0	7
E.J.N. Experiment Stn.	2	17	0	0	13
El Rodeo	3	21	0	0	4
Hacienda Los Ríos	0	0	0	0	12
La Gotera	0	0	0	0	10
Las Avispas	0	0	0	0	16
Marcela fragment	14	154	12	150	10
Q. La Duquesa	0	0	0	0	6
Quebrada Reventado (N)	0	0	0	0	10
Quebrada Reventado (S)	0	0	0	0	9
Sitio Cascante	0	0	0	0	10
Toronja fragment	12	117	6	71	10
Other fragments					
Canateca	0	0	0	0	10
Hacienda Tenorio	0	0	0	0	10
La Isla	5	81	5	81	9
Palmira	4	72	4	72	0
Paso Hondo	4	50	4	50	4
Salitral (Libertad)	0	0	0	0	11

[1] Sample sizes were higher for genetic diversity as progeny of indeterminate maternity were included, *i.e.* those collected beneath crowns of >1 mother tree. [2] Number of families, *i.e.* mother-trees; [3] Number of individual progeny

Table 5-3. Representation by block of seed sources (fragments) and open-pollinated families within fragments in a common garden experiment of *Anacardium excelsum*

Fragment/ Family	Block									
	1	2	3	4	5	6	7	8	9	10
El Cepo										
2	1	0	0	0	0	0	0	0	0	0
5	1	1	1	0	0	1	1	0	1	0
9	1	1	0	0	1	1	1	1	0	0
11	1	1	1	1	0	1	0	1	1	1
12	0	1	1	1	1	1	1	1	1	1
19	0	1	1	1	0	1	1	1	1	1
21	1	1	0	0	1	1	1	1	1	1
32	0	1	1	1	1	1	1	1	1	1
35	1	1	1	1	1	1	1	1	1	1
39	1	0	1	1	1	1	1	1	1	1
41	0	1	0	0	1	1	0	1	1	0
42	0	1	1	1	1	1	1	1	1	1
50	1	1	0	1	1	1	1	1	1	0
51	1	1	0	0	0	1	1	1	1	1
53	1	1	0	0	1	1	1	1	1	1
54	1	1	1	0	0	1	0	1	0	0
56	0	1	1	1	1	1	1	0	0	1
57	1	1	1	0	1	1	0	1	0	1
66	1	1	1	1	1	1	1	1	1	1
Bosque Duquesa										
700	1	0	1	1	1	1	1	1	0	0
701	1	1	1	1	0	1	1	1	1	1
706	0	0	0	0	1	0	1	0	0	1
700a	1	1	1	1	1	1	1	1	1	1
Toronja										
100	0	1	1	1	0	1	1	1	0	0
104	1	1	1	0	0	0	1	1	1	0
115	1	1	1	1	1	1	1	1	1	1
116	1	1	1	1	1	0	1	0	0	1
Marcela										
355	0	1	1	1	0	1	0	1	1	1
357	1	1	1	0	0	1	1	0	1	0
409	0	1	0	1	0	0	0	1	1	0
415	1	1	1	0	0	0	1	1	1	0
416	1	1	0	0	1	0	0	0	0	1
419	1	1	1	0	0	0	0	0	1	0
Congojas										
1	0	0	0	0	1	1	0	0	0	0
4	1	1	1	1	1	1	0	1	1	1
5	1	1	1	1	1	1	1	1	1	1
6	0	1	1	1	1	0	0	1	1	1
8	1	1	1	0	1	1	1	1	1	0
9	1	1	1	1	1	0	0	1	1	1
11	1	0	0	0	1	0	0	0	0	0
12	1	1	1	0	1	0	1	1	1	1
16	1	1	1	1	0	1	1	0	1	1

Table 5-3. Representation by block of seed sources (fragments) and open-pollinated families within fragments in a common garden experiment of *Anacardium excelsum* (continued)

Fragment/ Family	Block									
Congojas										
17	1	1	1	1	1	1	1	1	1	1
20	1	1	1	0	1	1	1	0	1	0
22	1	1	1	0	1	1	1	0	1	1
23	1	1	1	0	0	1	1	1	0	1
25	1	1	1	1	1	1	1	1	1	1
R.S.R.										
2	0	0	1	0	1	0	1	0	1	0
3	1	1	1	1	1	0	1	1	0	1
4	1	1	4	0	1	1	1	1	0	0
7	1	1	7	1	1	1	1	1	1	0
13	1	1	1	0	1	1	1	1	1	0
1000	0	1	1	1	0	1	1	1	1	0
2000	0	0	1	1	1	1	1	0	0	0
Ojoche										
10	1	0	0	0	1	1	0	0	0	1
12	1	1	0	1	1	0	0	1	1	1
16	0	1	0	0	1	1	0	1	1	1
19	1	0	1	0	0	1	1	1	0	1
21	1	1	1	1	1	1	1	1	0	0
Isla										
3	1	1	0	1	1	1	1	1	1	1
4	1	1	0	1	1	1	1	1	1	1
15	1	1	0	1	1	1		1	1	1
Palmira										
1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	
3	1	1	1	1	1	1	1	1	1	1
4	1	0	1	1	1	0	1	1	1	1

Table 5-4. Estimates of allele frequencies, allelic richness, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in populations of *Anacardium excelsum* from 10 forest fragments in northwestern Costa Rica, based on inferred maternal genotypes.

fragment / locus	allele frequencies			A[1]	H_e[2]	F_{is}[3]
	p_A[4]	q_B[4]	r_C[4]			
Bosque Duquesa						
AK2	0.88	0.12	n.a.	2	0.22	-0.14
LAP	0.75	0.25	0.0	2	0.38	-0.33
PGD	0.62	0.38	n.a	2	0.47	-0.60
PGM	0.75	0.25	n.a.	2	0.38	-0.33
UGPU	0.88	0.12	n.a	2	0.22	-0.14
means (S.D.)				2.0(.00)	0.33(0.11)	
El Cepo						
AK2	0.76	0.24	n.a.	2	0.36	0.02
LAP	0.82	0.13	0.05	3	0.30	-0.21
PGD	0.72	0.28	n.a.	2	0.40	-0.38*
PGM	0.31	0.69	n.a.	2	0.42	-0.44*
UGPU	0.82	0.18	n.a.	2	0.29	0.13
means (S.D.)				2.2 (.45)	0.35 (0.06)	
El Ojoche						
AK2	0.92	0.08	n.a.	2	0.15	-0.09
LAP	0.50	0.25	0.25	3	0.63	-1.0***
PGD	0.83	0.17	n.a.	2	0.29	-0.20
PGM	0.58	0.42	n.a.	2	0.49	-0.03
UGPU	0.82	0.18	n.a.	2	0.28	-0.20
means (S.D.)				2.20 (.45)	0.37 (0.19)	
La Isla						
AK2	0.62	0.37	n.a.	2	0.47	0.47
LAP	0.50	0.40	0.10	3	0.58	-0.20
PGD	0.70	0.30	n.a.	2	0.42	-0.43
PGM	0.50	0.50	n.a.	2	0.50	-0.20
UGPU	1.00	0.00	n.a.	1	0.00	M
means (S.D.)				2.0 (0.71)	0.39(0.23)	
Las Congojas						
AK2	0.76	0.24	n.a.	2	0.36	-0.31
LAP	0.84	0.16	0.00	2	0.26	-0.18
PGD	0.91	0.09	n.a.	2	0.16	-0.10
PGM	0.53	0.47	n.a.	2	0.50	-0.61*
UGPU	0.88	0.12	n.a.	2	0.21	-0.13
means (S.D.)				2.0 (0.00)	0.29 (.13)	

[1]Allelic richness; [2]Nei's expected heterozygosity (gene diversity); [3]fixation index. Asterisks indicate significance of associated G-test of Hardy-Weinberg equilibrium (*=0.05, **=0.01, ***≤0.001); M = monomorphic; [4]p,q,r = frequencies of alleles A, B, C, respectively

Table 5-4. Estimates of allele frequencies, allelic richness, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in populations of *Anacardium excelsum* from 10 forest fragments in northwestern Costa Rica, based on inferred maternal genotypes (continued)

fragment / locus	**allele frequencies**			A^1	H_e^2	F_{is}^3
	p_A^4	q_B^4	r_C^4			
Marcela						
AK2	0.96	0.03	n.a.	2	0.07	-0.04
LAP	0.46	0.54	0.0	2	0.50	-0.58*
PGD	0.85	0.14	n.a	2	0.24	0.42
PGM	0.61	0.39	n.a.	2	0.48	-0.05
UGPU	0.89	0.11	n.a	2	0.19	-0.12
means (S.D.)				2.0(.00)	0.29(0.19)	
Palmira						
AK2	0.25	0.75	n.a.	2	0.38	-0.33
LAP	0.50	0.50	0.0	2	0.50	-1.00*
PGD	0.25	0.75	n.a.	2	0.38	-0.33
PGM	0.12	0.88	n.a.	2	0.22	-0.14
UGPU	0.62	0.38	n.a.	2	0.47	-0.60
means (S.D.)				2.0 (.00)	0.39 (0.11)	
Paso Hondo						
AK2	0.25	0.75	n.a.	2	0.38	-0.33
LAP	0.75	0.25	0.0	2	0.38	-0.33
PGD	0.75	0.25	n.a.	2	0.38	-0.33
PGM	0.75	0.25	n.a.	2	0.38	-0.33
UGPU	1.00	0.00	n.a.	1	0.00	M
means (S.D.)				1.80 (.45)	0.30 (0.17)	
River Santa Rosa						
AK2	0.80	0.20	n.a.	2	0.32	-0.25
LAP	0.78	0.16	0.06	3	0.36	-0.29
PGD	0.75	0.25	n.a.	2	0.38	-0.33
PGM	0.15	0.85	n.a.	2	0.25	-0.18
UGPU	0.70	0.30	n.a.	2	0.42	0.05
means (S.D.)				2.2 (0.45)	0.35(0.06)	
Toronja						
AK2	0.93	0.07	n.a.	2	0.13	-0.08
LAP	0.25	0.58	0.17	3	0.57	0.55
PGD	0.57	0.43	n.a.	2	0.49	-0.75
PGM	0.43	0.57	n.a.	2	0.49	0.42
UGPU	1.00	0.00	n.a.	1	0.00	M
means (S.D.)				2.0 (0.71)	0.34 (.25)	

[1]Allelic richness; [2]Nei's expected heterozygosity (gene diversity); [3]fixation index. Asterisks indicate significance of associated G-test of Hardy-Weinberg equilibrium (*=0.05, **=0.01, ***≤0.001); M = monomorphic; [4]p,q,r = frequencies of alleles A, B, C, respectively

Table 5-5. Results of chi-square and likelihood ratio (G) tests of homogeneity of allele frequencies for five loci in populations of *Anacardium excelsum* from 10 forest fragments in northwestern Costa Rica, based on maternal genotypes inferred from progeny arrays (see text)

locus	chi-square, df, probability	G, df, probability
Overall		
AK	36.0, 9, <0.0001	33.6, 9, <0.0001
LAP	28.7, 9, <0.0001	28.8, 9, <0.0001
PGD	21.0, 9, 0.01	20.0, 9, 0.02
PGM	24.2, 9, 0.004	25.8, 9, 0.002
UGPU	13.7, 9, 0.13	16.6, 9, 0.05
Corobicí Group[1]		
AK	1.43, 3, 0.69	1.69, 3, 0.64
LAP	6.8, 3, 0.08	5.8, 3, 0.12
PGD	4.5, 3, 0.21	4.9, 3, 0.18
PGM	10.3, 3, 0.01	10.9, 3, 0.01
UGPU	2.87, 3, 0.41	2.71, 3, 0.44
Taboga group[1]		
AK	0.92, 2, 0.63	0.83, 2, 0.66
LAP	4.84, 2, 0.09	5.04, 2, 0.08
PGD	4.61, 2, 0.10	4.63, 2, 0.10
PGM	2.35, 2, 0.31	2.39, 2, 0.30
UGPU	1.72, 2, 0.42	2.78, 2, 0.25

1See text

Table 5-6. Estimates of Wright's statistics and *mN* for five loci of inferred maternal genotypes in populations of *Anacardium excelsum* from 10 forest fragments in northwestern Costa Rica

locus	sample size	F_{is}[1]	F_{it}[2]	F_{st}[3]	*mN*[4]
Overall					
AK	174	-0.0964	0.2400	0.3068	0.56
LAP	176	-0.3913	-02069	0.1325	1.63
PGD	182	-0.3692	-0.1632	0.1505	1.41
PGM	174	-0.1825	-0.0279	0.1779	1.15
UGPU	182	-0.1736	-0.0311	0.1214	1.81
Mean	**178**	**-0.2594**	**-0.0359**	**0.1775**	**1.16**
			Corobicí group		
AK	100	-0.1658	-0.1361	0.0255	9.6
LAP	102	-0.3557	-0.2570	0.0728	3.2
PGD	106	-0.2865	-0.2422	0.0344	7.0
PGM	98	-0.3294	-0.1586	0.1285	1.7
UGPU	106	-0.0205	0.0095	0.0294	8.2
Mean	**102**	**-0.2415**	**-0.1613**	**0.0646**	**3.6**
			Taboga group		
AK	50	-01047	-0.0839	0.0189	13.0
LAP	48	-0.2385	0.0814	0.1268	1.7
PGD	50	-0.4542	-0.3506	0.0712	3.3
PGM	50	0.0418	0.1106	0.0718	3.2
UGPU	50	-0.1322	-0.0839	0.0427	5.6
Mean	**50**	**-0.1866**	**-0.0878**	**0.0832**	**2.8**

[1]correlation between uniting gametes relative to the subpopulations; [2]correlation between uniting gametes relative to the population as a whole; [3]correlation between two gametes drawn randomly within subpopulations relative to that of two gametes drawn randomly from the population as a whole; [4]estimated number of migrants generation^{-1} (=0.25(1-F_{st})/F_{st})

Table 5-7. Pairwise estimates of Nei's unbiased genetic distance (below diagonal) and geographic distances (km, above diagonal) between ten populations of *Anacardium excelsum* located in northwestern Costa Rica, based on inferred maternal genotypes.

	Duquesa	**Cepo**	**Ojoche**	**Isla**	**Congojas**	**Marcela**	**Paso Hondo**	**Palmira**	**R.S.R.**	**Toronja**
Duquesa	0	17	17.25	7.9	16.75	2.75	9.6	24.75	15.25	4.5
Cepo	0.0533	0	2.92	9.25	1.85	18	11.1	8.1	3.1	19.3
Ojoche	0.0133	0.0457	0	11	2	21	13.75	6.35	3.6	21.5
Isla	0.0262	0.0367	0.0215	0	8.85	10.25	6.75	17.1	7.25	10.85
Congojas	0.0244	0.0197	0.0221	0.0298	0	19	11.85	8.25	1.7	19.4
Marcela	0.0281	0.0858	0.0063	0.0306	0.0491	0	12.5	27.3	17.25	5.8
Paso Hondo	0.3565	0.2211	0.4055	0.1934	0.3595	0.4182	0	19.3	11	9.6
Palmira	0.1	0.1439	0.1587	0.0451	0.0907	0.1813	0.259	0	10	27.8
R.S.R.	0.1111	0.0028	0.0736	0.0783	0.0534	0.115	0.2026	0.2315	0	18
Toronja	0.0739	0.1073	0.0442	0.0235	0.1272	0.0349	0.2754	0.2388	0.1293	0

Table 5-8. Estimates of allele frequencies, allelic richness, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in populations of *Anacardium excelsum* from 12 forest fragments in northwestern Costa Rica (progeny data)

fragment / locus	Allele frequencies			A^1	$\hat{A}_{ms}{}^2$	$H_e{}^3$	$F_{is}{}^4$
	$p_A{}^5$	$q_B{}^5$	$r_C{}^5$				
Bosque Duquesa							
AK*2*	0.90	0.10	n.a.	2	1.56	0.18	0.48***
LAP	0.77	0.23	0.0	2	1.88	0.35	-0.06
PGD	0.67	0.33	n.a	2	1.96	0.44	-0.01
PGM	0.73	0.27	n.a.	2	1.93	0.40	0.17
UGPU	0.94	0.06	n.a	2	1.41	0.11	0.17
means (S.D.)				2.0(.00)	1.75	0.30(0.14)	0.15
El Cepo							
AK*2*	0.72	0.28	n.a.	2	2.00	0.40	0.46***
LAP	0.8	0.06	0.05	3	2.72	0.21	0.22***
PGD	0.80	0.20	n.a.	2	2.00	0.32	0.25***
PGM	0.30	0.70	n.a.	2	2.00	0.42	0.22***
UGPU	0.84	0.16	n.a.	2	2.00	0.28	0.28***
means (S.D.)				2.2 (.45)	2.14	0.32 (0.09)	0.29
El Ojoche							
AK*2*	0.99	0.01	n.a.	2	1.14	0.02	-0.01
LAP	0.47	0.35	0.18	2	2.91	0.63	0.04
PGD	0.93	0.07	n.a.	2	1.59	0.13	0.04
PGM	0.60	0.40	n.a.	2	2.00	0.48	-0.11
UGPU	0.87	0.13	n.a.	2	1.82	0.23	0.28**
means (S.D.)				2.2 (.45)	1.89	0.30 (0.25)	0.05
El Rodeo	p_A	q_B	r_C				
AK*2*	0.48	0.52	n.a.	2	n.a.	0.50	0.04
LAP	0.98	0.02	0.0	2	n.a.	0.05	-0.02
PGD	0.95	0.05	n.a.	2	n.a.	0.09	-0.05
PGM	0.21	0.79	n.a.	2	n.a.	0.34	0.57**
UGPU	1.00	0.0	n.a.	1	n.a.	0.00	M
means (S.D.)				1.80 (.45)	n.a.	0.20 (0.21)	0.14
La Isla							
AK*2*	0.56	0.44	n.a.	2	2.00	0.49	0.59***
LAP	0.42	0.52	0.06	3	2.48	0.55	-0.05
PGD	0.79	0.21	n.a.	2	1.91	0.33	0.18
PGM	0.43	0.57	n.a.	2	2.00	0.49	0.17
UGPU	0.99	0.01	n.a.	2	1.12	0.02	-0.01
means (S.D.)				2.2 (.45)	1.90	0.38(0.21)	0.18

[1] Allelic richness; [2] rarefied allelic richness, i.e. standardized to the sample size used for estimation of maternal allelic richness; [3] Nei's expected heterozygosity (gene diversity); [4] fixation index. Asterisks indicate significance of associated G-test of Hardy-Weinberg equilibrium (*=0.05, **=0.01, ***≤0.001); M = monomorphic; [5] p,q,r = frequencies of alleles A, B, C, respectively

Table 5-8. Estimates of allele frequencies, allelic richness, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in populations of *Anacardium excelsum* from 12 forest fragments in northwestern Costa Rica (progeny data) (continued)

fragment / locus	**Allele frequencies**			A^{1}	$\hat{A}_{m}^{2}$	H_{e}^{3}	F_{is}^{4}
	p_{A}^{5}	q_{B}^{5}	r_{C}^{5}				
Las Congojas							
AK*2*	0.71	0.29	n.a.	2	2.00	0.41	0.44***
LAP	0.86	0.13	0.01	3	2.17	0.24	0.24***
PGD	0.92	0.08	n.a.	2	1.96	0.15	0.15*
PGM	0.68	0.32	n.a.	2	2.00	0.43	0.03
UGPU	0.85	0.15	n.a.	2	1.99	0.25	0.09
means (S.D.)				2.2 (.45)	2.02	0.30 (.12)	0.19
EJN							
AK*2*	0.72	0.28	n.a.	2	n.a.	0.40	-0.08
LAP	0.09	0.91	0.0	2	n.a.	0.16	-0.63*
PGD	0.97	0.03	n.a	2	n.a.	0.06	-0.03
PGM	0.47	0.53	n.a.	2	n.a.	0.50	-0.42
UGPU	1.00	0.0	n.a	1	n.a.	0.00	M
means (S.D.)				1.80(.45)	n.a.	0.22(0.22)	-0.29
Marcela							
AK*2*	0.94	0.06	n.a.	2	1.85	0.12	0.16
LAP	0.63	0.36	0.01	3	2.25	0.47	0.23**
PGD	0.91	0.09	n.a	2	1.94	0.17	0.37***
PGM	0.61	0.39	n.a.	2	2.00	0.48	0.08
UGPU	0.89	0.11	n.a	2	1.96	0.19	0.17
means (S.D.)				2.2 (.45)	2.00	0.28(0.18)	0.20
Palmira							
AK*2*	0.23	0.77	n.a.	2	1.88	0.35	0.39***
LAP	0.47	0.52	0.01	3	2.1	0.51	0.09
PGD	0.31	0.69	n.a.	2	1.95	0.43	0.41***
PGM	0.17	0.83	n.a.	2	1.75	0.29	0.08
UGPU	0.64	0.36	n.a.	2	1.97	0.46	0.48***
means (S.D.)				2.2 (.45)	1.93	0.41 (0.09)	0.29

[1] Allelic richness; [2] rarefied allelic richness, i.e. standardized to the sample size used for estimation of maternal allelic richness; [3] Nei's expected heterozygosity (gene diversity); [4] fixation index. Asterisks indicate significance of associated G-test of Hardy-Weinberg equilibrium (*=0.05, **=0.01, ***≤0.001); M = monomorphic; [5] p,q,r = frequencies of alleles A, B, C, respectively

Table 5-8. Estimates of allele frequencies, allelic richness, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in populations of *Anacardium excelsum* from 12 forest fragments in northwestern Costa Rica (progeny data) (continued)

population / locus	**Allele frequencies**			A^{1}	$\hat{A}_{ms}^{3}$	H_{e}^{3}	F_{is}^{4}
	p_{A}^{5}	q_{B}^{5}	r_{C}^{5}				
Paso Hondo							
AK*2*	0.25	0.75	n.a.	2	1.91	0.38	0.43**
LAP	0.87	0.11	0.02	2	1.72	0.23	0.34*
PGD	0.71	0.29	n.a.	2	1.94	0.41	0.83***
PGM	0.72	0.28	n.a.	2	1.94	0.40	-0.08
UGPU	0.99	0.01	n.a.	1	1.07	0.02	-0.01
means (S.D.)				2.2 (.45)	1.72	0.29 (0.17)	0.30
River Santa Rosa							
AK*2*	0.81	0.19	n.a.	2	1.99	0.31	0.17*
LAP	0.74	0.24	0.02	3	2.34	0.40	0.37***
PGD	0.81	0.19	n.a.	2	1.99	0.31	0.22**
PGM	0.19	0.81	n.a.	2	1.99	0.31	0.03
UGPU	0.74	0.26	n.a.	2	2.00	0.38	0.42***
means (S.D.)				2.2 (.45)	2.06	0.34(0.04)	0.24
Toronja							
AK*2*	0.86	0.15	n.a.	2	1.88	0.24	0.07
LAP	0.38	0.51	0.11	3	2.77	0.58	0.57***
PGD	0.65	0.35	n.a.	2	2.00	0.46	0.03
PGM	0.64	0.36	n.a.	2	2.00	0.46	0.11
UGPU	0.96	0.04	n.a.	2	1.48	0.07	-0.04
means (S.D.)				2.2 (.45)	2.03	0.36 (.20)	

[1] Allelic richness: [2] rarefied allelic richness, i.e. standardized to the sample size used for estimation of maternal allelic richness; [3] Nei's expected heterozygosity (gene diversity); [4] fixation index. Asterisks indicate significance of associated G-test of Hardy-Weinberg equilibrium (*=0.05, **=0.01, ***≤0.001); M = monomorphic; [5] p,q,r = frequencies of alleles A, B, C, respectively

Table 5-9. Results of chi-square and likelihood ratio (G) tests of homogeneity of allele frequencies for five loci in populations of *Anacardium excelsum* from 10 forest fragments in northwestern Costa Rica

locus	chi-square, df, probability	G, df, probability
Overall (excludes EJN, Rodeo)		
AK*2*	555.3,2, <0.00001	561.3, 2, <0.00001
LAP	459.8, 3, <0.00001	456.7, 3, <0.00001
PGD	365.8, 3, <0.00001	327.6, 3, <0.00001
PGM	416.8, 3, <0.00001	435.8, 3, <0.00001
UGPU	161.4, 3, <0.00001	175.9, 3, <0.00001
Corobicí group		
AK*2*	87.0, 3, <0.00001	123.2, 3, <0.00001
LAP	204.1, 3, <0.00001	175.6, 3, <0.00001
PGD	4.5, 3, <0.00001	4.9, 3, <0.00001
PGM	10.3, 3, <0.00001	10.9, 3, <0.00001
UGPU	2.87, 3, <0.00001	2.71, 3, <0.00001
Taboga group		
AK*2*	8.1, 2, 0.02	8.1, 2, 0.02
LAP	57.2, 2,<0.00001	58.2, 2, <0.00001
PGD	61.1, 2, <0.00001	65.9, 2, <0.00001
PGM	5.7, 2, 0.06	5.8, 2, 0.05
UGPU	9.2, 2, 0.01	9.4, 2, 0.01

Table 5-10. Estimates of Wright's statistics and *mN* for five loci of progeny genotypes in populations of *Anacardium excelsum* from 10 forest fragments in northwestern Costa Rica.

Locus	Sample size	F_{is}[1]	F_{it}[2]	F_{st}[3]	*mN*[4]
AK	2792	0.3905	0.5811	0.3127	0.55
LAP	2712	0.1989	0.3104	0.1392	1.55
PGD	2926	0.2610	0.3796	0.1604	1.31
PGM	2686	0.0459	0.2059	0.1676	1.24
UGPU	2934	0.2938	0.3633	0.0983	2.29
Mean	**2810**	**0.2148**	**0.3563**	**0.1803**	**1.14**
Corobicí group					
AK	1728	0.3690	0.4185	0.0785	2.9
LAP	1680	0.2019	0.3169	0.1442	1.5
PGD	1822	0.1919	0.2161	0.0300	8.1
PGM	1600	0.0378	0.1967	0.1652	1.3
UGPU	1836	0.2838	0.2949	0.0156	15.8
Mean	**1733**	**0.2148**	**0.2847**	**0.0981**	**2.3**
Taboga group					
AK	676	0.2284	0.2360	0.0099	25.1
LAP	636	0.2775	0.3578	0.1111	2.0
PGD	680	0.0690	0.1373	0.0734	3.2
PGM	684	0.0430	0.0531	0.0105	23.6
UGPU	672	0.1294	0.1401	0.0123	20.0
Mean	**670**	**0.1438**	**0.1908**	**0.0549**	**4.3**

[1]correlation between uniting gametes relative to the subpopulations; [2]correlation between uniting gametes relative to the population as a whole; [3]correlation between two gametes drawn randomly within subpopulations relative to that of two gametes drawn randomly from the population as a whole; [4]estimated number of migrants generation^{-1} ($=0.25(1-F_{st})/F_{st}$)

Table 5-11. Pairwise estimates of Nei's unbiased genetic distance (below diagonal) and geographic distances (km, above diagonal) between ten populations of *Anacardium excelsum* located in northwestern Costa Rica (progeny data)

	Duquesa	**Cepo**	**Ojoche**	**Isla**	**Congojas**	**Marcela**	**Paso Hondo**	**Palmira**	**R.S.R.**	**Toronja**
Duquesa	0	17	17.25	7.9	16.75	2.75	9.6	24.75	15.25	4.5
Cepo	0.0771	0	2.92	9.25	1.85	18	11.1	8.1	3.1	19.3
Ojoche	0.0481	0.0946	0	11	2	21	13.75	6.35	3.6	21.5
Isla	0.1024	0.0881	0.0831	0	8.85	10.25	6.75	17.1	7.25	10.85
Congojas	0.0317	0.0471	0.0583	0.0923	0	19	11.85	8.25	1.7	19.4
Marcela	0.0257	0.0691	0.0082	0.0705	0.0313	0	12.5	27.3	17.25	5.8
Paso Hondo	0.3893	0.2561	0.4645	0.1839	0.3888	0.4204	0	19.3	11	9.6
Palmira	0.1295	0.1304	0.2342	0.1129	0.0802	0.1794	0.2522	0	10	27.8
R.S.R.	0.1072	0.0158	0.085	0.0854	0.0864	0.0702	0.2359	0.2165	0	18
Toronja	0.0384	0.1311	0.0361	0.0524	0.0935	0.0359	0.3173	0.1896	0.1249	0

Table 5-12. Estimates of current (1999) gene flow into the Paso Hondo population based on two alleles absent from the Paso Hondo population

Allele	**q_s^1**	**q_t^2 1998**	**m^3**
UGPU-B	0.15	0.01	0.07
LAP-C	0.07	0.02	0.28
Mean			0.18

[1]allele frequency in source population, *i.e.* mean allele frequency over all populations; [2]allele frequency in Paso Hondo progeny; [3]m=proportion of immigrant alleles, i.e. q_t/q_s (see text)

Table 5-13. Estimates of mating system parameters of *A. excelsum* in populations located in 10 forest fragments in northwestern Costa Rica

population	$\hat{F}_e$[1]	t_m (s.e.)[2]	t_s (s.e.)[3]	t_m-t_s	r_t[4]	r_p[5]
Bosque Duquesa	0.40	0.430* (0.199)	0.647* (0.170)	-0.217* (0.044)	0.124* (0.060)	0.418* (0.121)
El Cepo	0.28	0.566* (0.078)	0.656* (0.068)	-0.090* (0.018)	0.220*(0.088)	0.878*(0.026)
El Ojoche	0.15	0.744* (0.106)	0.709* (0.093)	0.036 (0.049)	0.108 (0.080)	0.866* (0.055)
La Isla	0.43	0.396* (0.222)	0.656* (0.188)	-0.260* (0.058)	0.161* (0.063)	0.665* (0.068)
Las Congojas	0.15	0.739* (0.094)	0.811* (0.073)	-0.073* (0.028)	0.139* (0.045)	0.766* (0.064)
Marcela	0.19	0.684*(0.092)	0.726* (0.074)	-0.042(0.034)	0.093* (0.023)	0.469* (0.116)
Palmira	0.58	0.266* (0.088)	0.258* (0.083)	0.008 (0.015)	0.230* (0.102)	0.868* (0.040)
Paso Hondo	0.26	0.581* (0.193)	0.665* (0.186)	-0.084 (0.042)	0.154 (0.307)	0.978* (0.087)
River Santa Rosa	0.40	0.427* (0.085)	0.487* (0.064)	-0.060* (.025)	0.101 (0.052)	0.480* (0.088)
Toronja	0.22	0.638* (0.089)	0.668* (0.069)	-0.030 (0.027)	0.092 (0.111)	0.980* (0.012)

[1]expected equilibrium value of F (see text);[2]estimated multilocus outcrossing rate, * indicates significant estimate, *i.e.* $t_m+2s_x<1.0$); [3]average of estimated single-locus outcrossing rate; [4]estimated correlation of outcrossing rates, * indicates significant estimate, *i.e.* $r> 2$s.e.; [5]estimated correlation of outcrossed paternity

Table 5-14. Mean population flowering indices and associated data in 24 forest fragments of *Anacardium excelsum* in northwestern Costa Rica.

Fragment	mean number of panicles tree^{-1}	mean dbh of sampled trees	standard deviation of sampled trees	Shannon's equitability (J) for flowering
Fragments of type 1 (sheltered, on watercourses)				
Canateca	133.4	112.4	15.8	0.81
El Cepo	137.5	136.4	26.0	0.85
El Ojoche	184.9	81.6	16.4	0.74
Finca La Gotera	53.2	113.4	31.4	0.77
Hcda. Tenorio	67.3	91.2	18.9	0.83
Las Congojas	30.4	84.7	17.1	0.44
Q. Reventado (south)	121.9	132.7	18.9	0.76
Quebrada El Lajero	94.9	107.3	21.4	0.80
River Santa Rosa	136.0	120.2	38.8	0.62
Other fragments (type 0)				
Bosque La Duquesa	18.0	104.2	29.3	0.41
COSTASEM	2.14	100.9	25.8	0.55
EJN	10.4	91.1	29.2	0.52
El Rodeo	211.2	114.2	34.6	0.61
Hacienda Los Ríos	54.9	96.1	43.5	0.48
La Isla	38.1	104.9	18.4	0.54
Las Avispas	1.9	97.8	37.1	0.65
Marcela	40.6	153.4	33.9	0.68
Oldemar	20.3	98.1	23.3	0.71
Paso Hondo	170.2	151.2	19.6	0.66
Q. Reventado (north)	41.6	104.9	21.6	0.67
Quebrada La Duquesa	33.8	124.0	32.4	0.17
Salitral-Libertad	13.1	93.4	34.6	0.41
Sitio Cascante	30.8	102.3	23.3	0.69
Toronja	66.6	101.9	19.1	0.62

Table 5-15. Linear regression of fragment mean number of panicles tree^{-1} on fragment type and mean dbh of sampled trees in 24 fragment populations of *A. excelsum* in northwestern Costa Rica.

Source of variation	degrees of freedom	Mean square	F	significance[1] R^2
Regression	2	14691.0	5.24	p= .014, R^2=0.33
Residual	21	2800.8		
Total	23			
	parameter estimates	**std error**	**t**	**significance[1]**
intercept	-.78.18	64.82	-1.21	0.24
type	56.77	22.31	2.54	0.018
mean dbh	1.18	0.58	2.03	0.056

Table 5-16. Linear regressions of flowering equitability on fragment type and mean dbh and equitability on type and dbh standard deviation of sampled trees in 24 fragment populations of *A. excelsum* in northwestern Costa Rica.

Source of variation	**degrees of freedom**	**Mean square**	***F***	**significance[1], R^2**
Equitability v. type and mean dbh				
Regression	2	0.098	5.02	p= .016, R^2=0.32
Residual	21	0.020		
Total	23			
	parameter estimates	**std error**	**t**	**significance**
intercept	0.39	0.17	2.29	0.032
type	0.18	0.06	3.02	0.006
mean dbh	0.002	0.001	0.99	0.330
Equitability v. type and standard deviation of dbh				
Regression	2	0.103	5.40	p= .013, R^2=0.34
Residual	21	0.019		
Total	23			
	parameter estimates	**std error**	**t**	**significance**
intercept	0.69	0.11	6.03	<0.0001
type	0.15	0.06	2.43	0.024
dbh *s*	-0.005	0.004	-1.23	0.232

Table 5-17. Analysis of variance of effect of origin (fragment) on weight of seeds collected from seven populations of *Anacardium excelsum* located in northwestern Costa Rica.

source of variation	degrees of freedom	mean square	*F*	*p*
Fragment	6	57603.14	35.56	p<0.0001
Residual	378	1619.79		
Total	384			

Table 5-18. Means, standard errors and Bonferroni groupings of weight of seeds collected from seven populations of *Anacardium excelsum* located in northwestern Costa Rica.

Fragment	Mean weight (g), Bonferroni grouping[1]		Standard error
El Cepo	A	2.59	0.0546
Canateca	A	2.49	0.0641
Marcela	B	2.19	0.0480
Quebrada Reventado	CB	2.10	0.0549
Toronja	CD	1.92	0.0412
Salitral	CD	1.87	0.0579
Duquesa	D	1.72	0.0561

[1]Same letter indicates means not significantly different, p=0.05

Table 5-19. Analysis of variance of effect of origin (fragment) on arcsin germination percentage, four-month height and number of days to germination of seedlings from seven populations of *Anacardium excelsum* located in northwestern Costa Rica.

trait / source	degrees of freedom	mean squares (type 3)	*F*	*p*
Height, four months				
Block	4	121.04	3.08	0.02
Fragment	6	270.27	6.88	<0.0001
error	125	43.78		
Germination day				
Block	4	2.83	0.71	0.59
Fragment	6	9.72	2.44	0.03
Error	124			
Arcsin Germination percentage				
Block	4	0.01	0.40	0.81
Fragment	6	0.35	9.41	<0.0001
Error	24	0.037		

Table 5-20. Germination percentage, least square means for height at four months (cm) and number of days to germination for *Anacardium excelsum* seedlings from seven forest fragments in northwestern Costa Rica

Fragment	Germination percentage[1]	Fragment	Height, 4 months (cm) (S.E.)	Fragment	germination day (S.E.)	Fragment	Height (with covariates), 4 months (cm) S.E.
Marcela	A 78.2	Marcela	A 31.5 (1.05)	Reventado	A 14.2 (0.56)	Marcela	A 30.8 (0.98)
El Cepo	B A 58.2	El Cepo	B A 29.4 (1.28)	Marcela	A 14.5 (0.34)	El Cepo	B A 28.4 (1.28)
Canateca	B A 54.5	Canateca	C B 25.6 (1.21)	Toronja	A 14.6 (0.61)	Canateca	B 25.7 (1.20)
Duquesa	B 44.4	Salitral	C B A 25.0 (2.39)	El Cepo	A 15.4 (0.40)	Duquesa	B 25.5 (1.51)
Toronja	B 31.0	Reventado	C B 23.4 (1.82)	Canateca	A 15.7 (0.39)	Salitral	B A 23.7 (2.63)
Reventado	B 27.2	Duquesa	C 23.0 (1.46)	Salitral	A 16.0 (0.48)	Reventado	B 23.4 (1.78)
Salitral	B 21.8	Toronja	C 22.1 (1.90)	Duquesa	A 16.0 (0.83)	Toronja	B 22.2 (1.77)

[1] Data presented are overall, untransformed experimental means. Bonferroni grouping based on ANOVA of arcsin transformed data (see Table 5-19)

Table 5-21. Analysis of covariance of effect of origin (fragment), seed weight and germination day on height of four-month old seedlings from seven populations of *Anacardium excelsum* located in northwestern Costa Rica.

trait / source	degrees of freedom	mean squares (type 3)	*F*	*p*
Height, four months				
Block	4	57.6	1.7	0.15
Fragment	6	172.4	5.2	<0.0001
germination day	1	755.9	22.6	<0.0001
seed weight	1	183.0	5.48	0.02
error	119	33.4		

Table 5-22. Analysis of variance of a greenhouse experiment comparing growth of *Anacardium excelsum* seedlings from 9 forest fragments in northwestern Costa Rica

trait / source	df	expected mean squares	mean squares (type 3)	*F*	*p*
Height, 81 days					
Block	9	$\sigma^2_{\text{error}}+43.222\sigma^2_{\text{block}}$	35.78	4.12	<0.0001
Fragment	8	$\sigma^2_{\text{error}}+5.9104\sigma^2_{\text{fam(frag)}}+Q_{\text{frag}}$	46.94	2.09[1]	0.05
Family-in-fragment	58	$\sigma^2_{\text{error}}+6.6671\sigma^2_{\text{fam(fragment)}}$	22.51	2.59	<0.0001
Error	380		8.68		
Height, 167 days					
Block	9	$\sigma^2_{\text{error}}+43.333\sigma^2_{\text{block}}$	43.7	2.1	0.03
Fragment	8	$\sigma^2_{\text{error}}+5.958\sigma^2_{\text{fam(frag)}}+Q_{\text{frag}}$	114.9	2.06[1]	0.05
Family-in-fragment	58	$\sigma^2_{\text{error}}+6.6803\sigma^2_{\text{fam(frag)}}$	55.7	2.68	0.0009
Error	381		20.8		
Diameter, 167 days					
Block	9	$\sigma^2_{\text{error}}+43.333\sigma^2_{\text{block}}$	4.7	2.7	0.005
Fragment	8	$\sigma^2_{\text{error}}+5.959\sigma^2_{\text{fam(frag)}}+Q_{\text{frag}}$	5.8	1.49[1]	0.18
Family-in-fragment	58	$\sigma^2_{\text{error}}+6.6803\sigma^2_{\text{fam(frag)}}$	3.90	2.26	<0.0001
Error	381		1.73		
Testa length					
Block	9	$\sigma^2_{\text{error}}+56.222\sigma^2_{\text{block}}$	9.6	1.0	0.44
Fragment	8	$\sigma^2_{\text{error}}+8.225\sigma^2_{\text{fam(frag)}}+Q_{\text{frag}}$	43.8	1.45[1]	0.20
Family-in-fragment	56	$\sigma^2_{\text{error}}+8.7419\sigma^2_{\text{fam(frag)}}$	30.2	3.2	<0.0001
Error	497		9.6		

[1] Approximate F-test based on family-in-fragment mean square as denominator

Table 5-23. Least square means of total height at 81 and 167 days, diameter 2cm above root collar at 167 days and testa length of *Anacardium excelsum* seedlings from 9 forest fragments in northwestern Costa Rica

Fragment	Height, 81 days (cm) ($s_{\bar{x}}$)	Fragment	Diameter, 167 days (mm) ($s_{\bar{x}}$)	Fragment	Height at 167 days (cm) ($s_{\bar{x}}$)	Fragment	Testa length (mm) ($s_{\bar{x}}$)
Marcela	18.3 (0.91)	Marcela	7.5 (0.38)	Marcela	25.0 (1.43)	R.S.R.	32.4 (0.77)
Congojas	17.6 (0.60)	Congojas	7.1 (0.25)	Congojas	23.6 (0.95)	Congojas	32.3 (0.54)
La Isla	17.3 (0.95)	El Ojoche	7.0 (0.37)	Isla	22.4 (1.47)	Isla	32.0 (1.06)
Palmira	16.6 (0.79)	Isla	7.0 (0.39)	Palmira	22.3 (1.25)	El Cepo	31.8 (0.43)
El Cepo	15.8 (0.48)	Palmira	6.9 (0.33)	Toronja	20.8 (1.52)	Ojoche	31.7 (0.91)
El Ojoche	15.7 (0.83)	Toronja	6.6 (0.40)	El Cepo	20.8 (0.75)	Marcela	31.4 (0.76)
Toronja	15.7 (1.00)	El Cepo	6.5 (0.20)	R.S.R.	20.4 (1.31)	Palmira	31.2 (0.90)
R.S.R.	15.5 (0.83)	B. Duquesa	6.5 (0.42)	Ojoche	20.2 (1.40)	Toronja	29.8 (0.94)
B. Duquesa	14.6 (1.01)	R.S.R.	6.2 (0.35)	B. Duquesa	19.2 (1.48)	B. Duquesa	29.5 (0.98)

Table 5-24. Analysis of covariance of a greenhouse experiment comparing growth of *Anacardium excelsum* seedlings from 9 forest fragments in northwestern Costa Rica

trait / source	df	expected mean squares	mean squares (type 3)	*F*	*p*
Height, 81 days					
Block	9	$\sigma^2_{error}+39.385\sigma^2_{block}$	22.2	3.51	<0.0004
Fragment	8	$\sigma^2_{error}+5.4588\sigma^2_{fam(frag)}+Q_{frag}$	36.3	2.29	0.03
Family-in-fragment	56	$\sigma^2_{error}+6133\sigma^2_{fam(frag)}$	15.9	2.52[1]	<0.0001
testa length	1	$\sigma^2_{error}+Q_{length}$	475.7	75.4	<0.0001
testa shedding date	1	$\sigma^2_{error}+Q_{shed}$	615.4	97.5	<0.0001
Error	346		6.31		
Height, 167 days					
Block	9	$\sigma^2_{error}+39.945\sigma^2_{block}$	17.7	1.17	0.3129
Fragment	8	$\sigma^2_{error}+5.5001\sigma^2_{fam(frag)}+Q_{frag}$	84.4	2.21[1]	0.04
Family-in-fragment	56	$\sigma^2_{error}+6.3255\sigma^2_{fam(frag)}$	38.2	2.53	<0.0001
testa length	1	$\sigma^2_{error}+Q_{length}$	1109.0	73.5	<0.0001
testa shedding date	1	$\sigma^2_{error}+Q_{shed}$	1562.5	103.5	<0.0001
Error	347		15.1		
Diameter, 167 days					
Block	9	$\sigma^2_{error}+39.945\sigma^2_{block}$	2.1	1.59	0.12
Fragment	8	$\sigma^2_{error}+5.5001\sigma^2_{fam(frag)}+Q_{frag}$	4.4	1.73[1]	0.11
Family-in-fragment	56	$\sigma^2_{error}+6.3255\sigma^2_{fam(frag)}$	2.54	1.92	0.0002
testa length	1	$\sigma^2_{error}+Q_{length}$	34.1	25.9	<0.0001
testa shedding date	1	$\sigma^2_{error}+Q_{shed}$	168.4	127.5	<0.0001
Error	347		1.73		

[1] Approximate F-test based on family-in-fragment mean square as denominator

Table 5-25. Least squares means of total height at 81 and 167 days and diameter 2cm above root collar at 167 days of *Anacardium excelsum* seedlings from 9 forest fragments in northwestern Costa Rica (based on analysis of covariance)

Fragment	Height, 81 days ($S_{\bar{x}}$) (cm)	Fragment	Diameter, 167 days ($S_{\bar{x}}$) (mm)	Fragment	Height at 167 days ($S_{\bar{x}}$) (cm)
Congojas	17.4 (0.46)	El Ojoche	7.1 (0.31)	Marcela	23.4 (1.23)
Marcela	17.3 (0.79)	Congojas	7.0 (0.18)	Congojas	23.1 (0.72)
La Isla	16.9 (0.86)	Marcela	7.0 (0.32)	El Cepo	22.0 (0.61)
El Cepo	16.6 (0.39)	Isla	6.9 (0.33)	Palmira	22.0 (1.09)
Palmira	16.5 (0.71)	Palmira	6.8 (0.28)	Isla	21.7 (1.30)
El Ojoche	16.1 (0.79)	El Cepo	6.8 (0.16)	Ojoche	20.8 (1.22)
Toronja	15.7 (0.83)	Toronja	6.4 (0.33)	Toronja	20.8 (1.29)
R.S.R.	14.8 (0.82)	B. Duquesa	6.1 (0.36)	R.S.R.	19.1 (1.27)
B. Duquesa	14.0 (0.91)	R.S.R.	6.0(0.32)	B. Duquesa	18.2 (1.41)

Table 5-26. Numbers of albino and normal seedlings in progeny of three trees of *Anacardium excelsum* from populations located in forest fragments in northwestern Costa Rica

Family	Number of seed sown	Number of albino germinants	Number of normal germinants
1505	75	0	19
1541	45	1	24
Ojoche 21	70	13	31

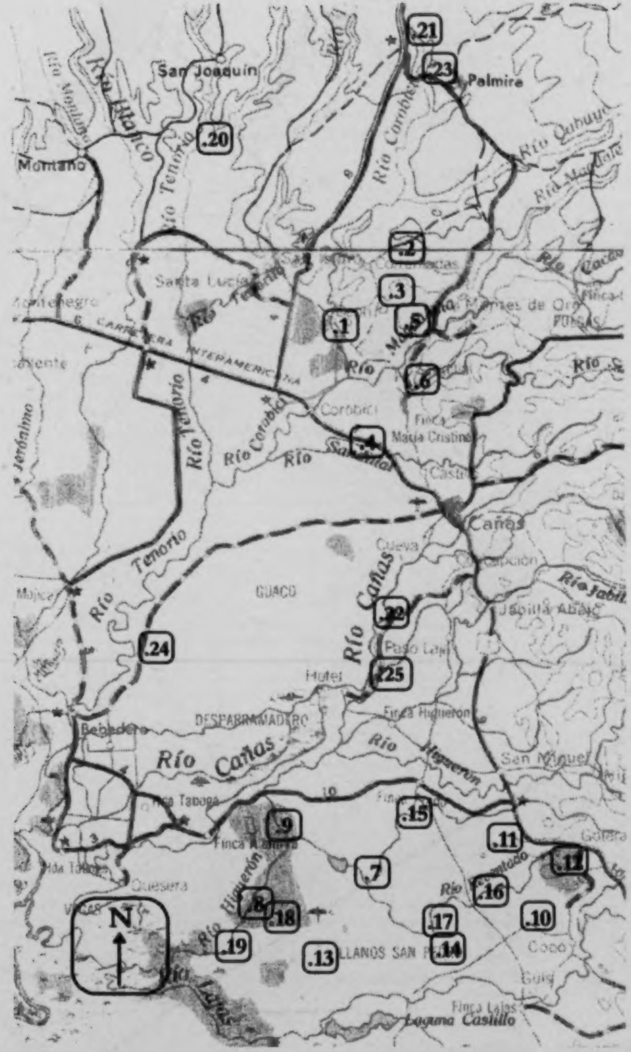
.21
.23
Palmire
San Joaquín
.20
Montano
Río Tenorio
Río Corobicí
.2
.3
.1
.5
.6
Santa Lucía
Carretera Interamericana
Río Corobicí
Río Tenorio
Finca María Cristina
.4
Cañas
Cueva
GUACO
Río Tenorio
Río Cañas
.22
.24
.25
Hotel
Paso Lajas
Finca Higuerón
Desparramadero
Bebedero
Río Cañas
Río Higuerón
San Miguel
.15
.9
.11
.12
.7
.16
.8
.18
.17
.10
.19
.13
.14
Llanos San
Quesera
Coco
Finca Lajas
Laguna Castillo
N

Figure 5-1. Populations sampled in a study of genetic effects of forest fragmentation on *Anacardium excelsum* populations located near Cañas, Guanacaste province, Costa Rica. Periods mark population centres. Key to populations: 1: El Cepo; 2: El Ojoche; 3: Las Congojas; 4: Oldemar; 5: Quebrada Lajero; 6: River Santa Rosa; 7: Bosque Duquesa; 8: COSTASEM; 9: E.J.N.; 10: El Rodeo; 11: Hacienda Los Rios; 12: La Gotera; 13: Las Avispas; 14: Marcela; 15: Quebrada Duquesa; 16: Río Reventado (north); 17: Río Reventado (south); 18: Sitio Cascante; 19: Toronja; 20: Canateca; 21: Hacienda Tenorio; 22: La Isla; 23: Palmira; 24: Paso Hondo; 25: Quebrada Salitral (Libertad). Scale: 1:200000. Shading (on original) indicates wooded areas, but does not include all wooded areas (source: IGN 1988a, 1988b, reproduced by permission; see Appendix 4).

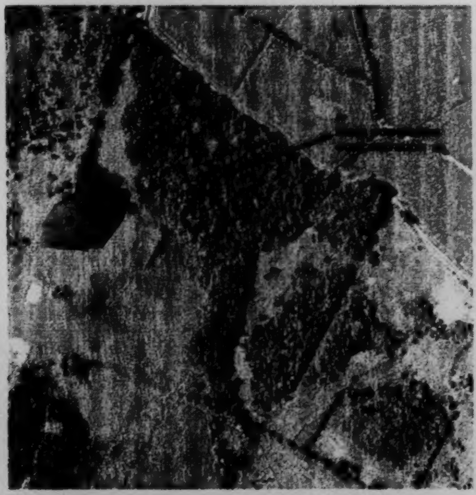

Figure 5-2. Location of Bosque Duquesa tree 706 in relation to other sampled trees (scale approximately 1:7400) (photo: Aerofotos de Costa Rica S.A.)

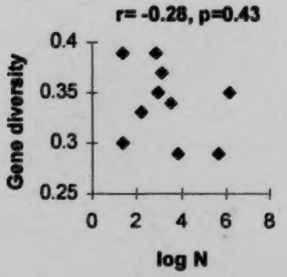

Figure 5-3. Scatter plot of log population size (log N) and gene diversity of adult trees in ten populations of *Anacardium excelsum* located in forest fragments in northwestern Costa Rica.

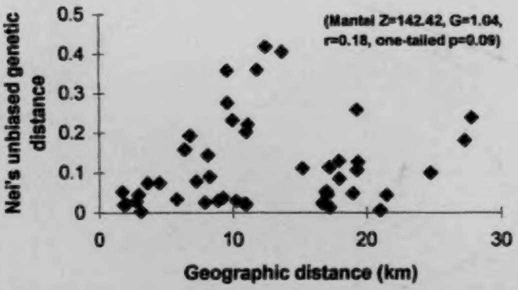

Figure 5-4. Scatter plot of pairwise relationship between geographic and Nei's unbiased genetic distances in 10 populations of *Anacardium excelsum* located in northwestern Costa Rica (based on inferred maternal genotypes).

```
                                                           +---Bosque Duquesa
                                                        +--3
                                                        !  !  +El Ojoche
                                                     +--5  +--2
                                                     !  !     +Marcela
                                                     !  !
                                                +----6  +------Las Congojas
                                                !    !
                                                !    !     +---La Isla
                                 +--------------7    +-----4
                                 !              !          +---Toronja
                                 !              !
  +------------------------------8              !             +El Cepo
  !                              !              +-------------1
  !                              !                            +River Santa Rosa
--9                              !
  !                              +-----------------------------Paso Hondo
  !
  +------------------------------------------------------------Palmira
```

Figure 5-5. UPGMA dendrogram based on Nei's unbiased genetic distance between maternal trees in ten populations of *A. excelsum* located in northwestern Costa Rica.

```
                                              +-El Cepo
  +-------------------------------------------1
  !                                           +-River Santa Rosa
--3
  !                        +--------------------El Ojoche
  +------------------------2
                           +--------------------Las Congojas
```

Figure 5-6. UPGMA dendrogram based on Nei's unbiased genetic distance between maternal trees in four populations of *A. excelsum* (Corobicí group) located in northwestern Costa Rica.

```
                         +------------------------Bosque Duquesa
  +----------------------1
--2                      +------------------------Marcela
  !
  +-----------------------------------------------Toronja
```

Figure 5-7. UPGMA dendrogram based on Nei's unbiased genetic distance between maternal trees in three populations of *A. excelsum* (Taboga group) located in northwestern Costa Rica.

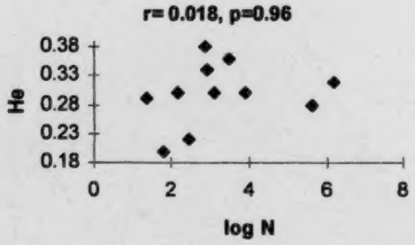

Figure 5-8. Scatter plot of log population size (log N) and gene diversity of progeny in twelve populations of *Anacardium excelsum* located in forest fragments in northwestern Costa **Rica**

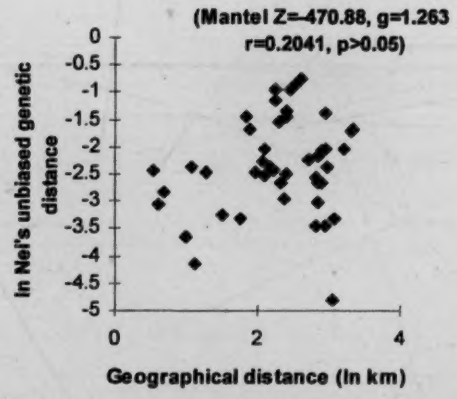

Figure 5-9. Scatter plot of pairwise relationship between geographic and Nei's unbiased genetic distances in 10 populations of *Anacardium excelsum* located in northwestern Costa Rica (progeny genotypes)

```
                                               !   +-----Bosque Duquesa
                                               +---3
                                               !   +-----Las Congojas
                                       +-------7
                                       !       !       +El Ojoche
                                       !       !  +----1
                                       !       +--4    +Marcela
                                   +---8          !
                                   !   !          +-----Toronja
                                   !   !
                                   !   !          +------La Isla
                           +-------9   +----------5
                           !       !              +------EJN
                           !       !
                           !       !                   +--El Cepo
  +-----------------------10       !             +-----2
  !                        !       +-------------6     +--R. Santa Rosa
  !                        !                     !
-11                        !                     +-------El Rodeo
  !                        !
  !                        +-----------------------------Paso Hondo
  !
  +------------------------------------------------------Palmira
```

Figure 5-10. UPGMA dendrogram based on Nei's unbiased genetic distance between progeny in 12 populations of *Anacardium excelsum* located in northwestern Costa Rica.

```
                                           +-------El Cepo
              +----------------------------1
  +-----------2                            +-------R. Santa Rosa
  !           !
--3           +------------------------------------Las Congojas
  !
  +------------------------------------------------El Ojoche
```

Figure 5-11. UPGMA dendrogram based on Nei's unbiased genetic distance between progeny in four populations of *A. excelsum* (Corobicí group) located in northwestern Costa Rica.

```
                                                 +------Bosque Duquesa
                                            +----1
                       +--------------------2    +------Marcela
                       !                    !
  +--------------------3                    +-----------Toronja
  !                    !
--4                    +--------------------------------EJN
  !
  +-----------------------------------------------------El Rodeo
```

Figure 5-12. UPGMA dendrogram based on Nei's unbiased genetic distance between progeny in three populations of *Anacardium excelsum* (Taboga group) located in northwestern Costa Rica.

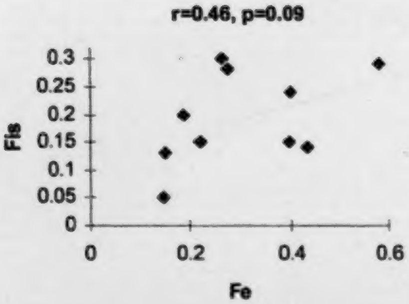

Figure 5-13. Scatter plot of observed inbreeding coefficients (F_{is}) and equilibrium inbreeding coefficients (F_e) based on estimated outcrossing rates ($\hat{F}_e = (1\text{-}t_m)/(1+t_m)$) (one-tailed) in populations of *Anacardium excelsum* located in 10 forest fragments in northwestern Costa Rica (p-value is one-tailed)

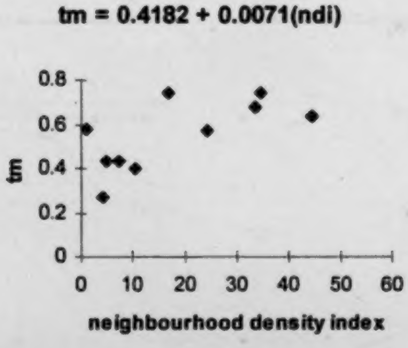

Figure 5-14. Linear regression of multilocus population outcrossing rates on population mean neighborhood density index (see text) in ten populations of *Anacardium excelsum* located in northwestern Costa Rica

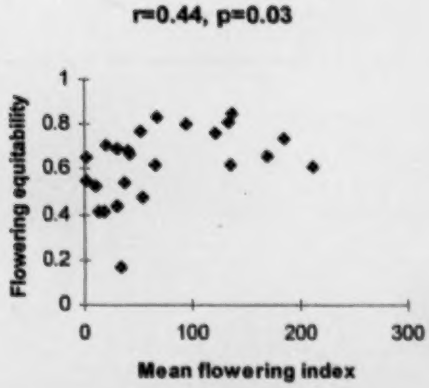

Figure 5-15. Scatter plot of flowering equitability and mean flowering index in 24 populations of *Anacardium excelsum* located in forest fragments in northwestern Costa Rica.

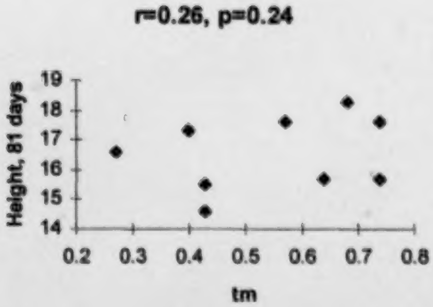

Figure 5-16. Scatter plot of least square mean height at 81 days (based on ANOVA) and estimated outcrossing rate in populations of *Anacardium excelsum* from nine forest fragments in northwestern Costa Rica (p-value is one-tailed)

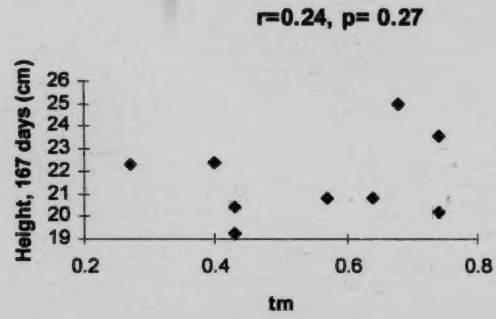

Figure 5-17. Scatter plot of least square mean height at 167 days (based on ANOVA)and estimated outcrossing rate in populations of *Anacardium excelsum* from nine forest fragments in northwestern Costa Rica (p-value is one-tailed)

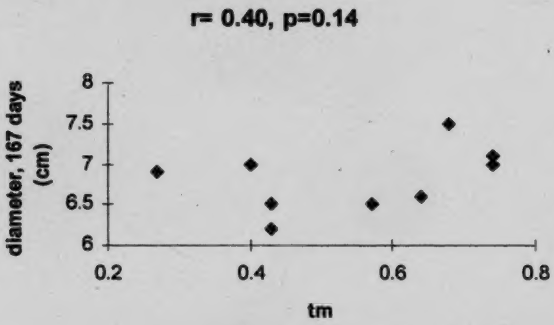

Figure 5-18. Scatter plot of least square mean diameter at 167 days (based on ANOVA) and estimated outcrossing rate in populations of *Anacardium excelsum* from nine forest fragments in northwestern Costa Rica (p-value is one-tailed)

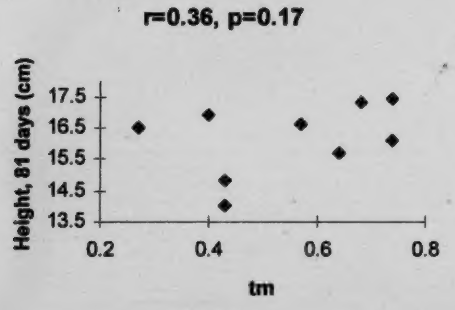

Figure 5-19. Scatter plot of least square mean height at 81 days (based on ANCOVA) and estimated outcrossing rate in populations of *Anacardium excelsum* from nine forest fragments in northwestern Costa Rica (p-value is one-tailed)

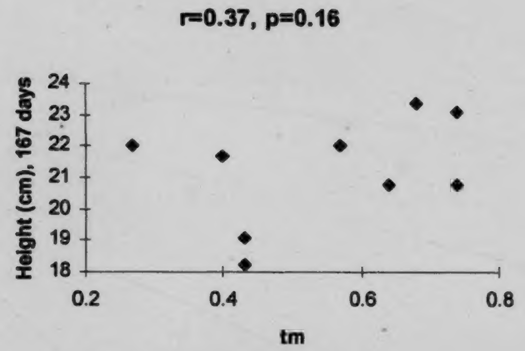

Figure 5-20. Scatter plot of least square mean height at 167 days (based on ANCOVA) and estimated outcrossing rate in populations of *Anacardium excelsum* from nine forest fragments in northwestern Costa Rica (p-value is one-tailed).

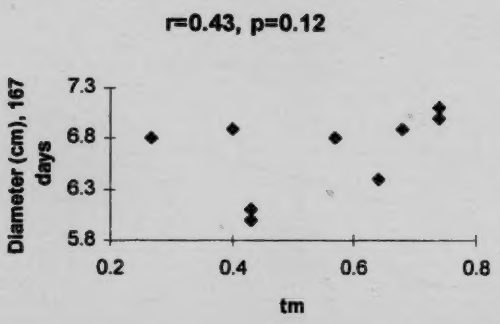

Figure 5-21. Scatter plot of least square mean diameter at 167 days (based on ANCOVA) and estimated outcrossing rate in populations of *Anacardium excelsum* from nine forest fragments in northwestern Costa Rica (p-value is one-tailed)

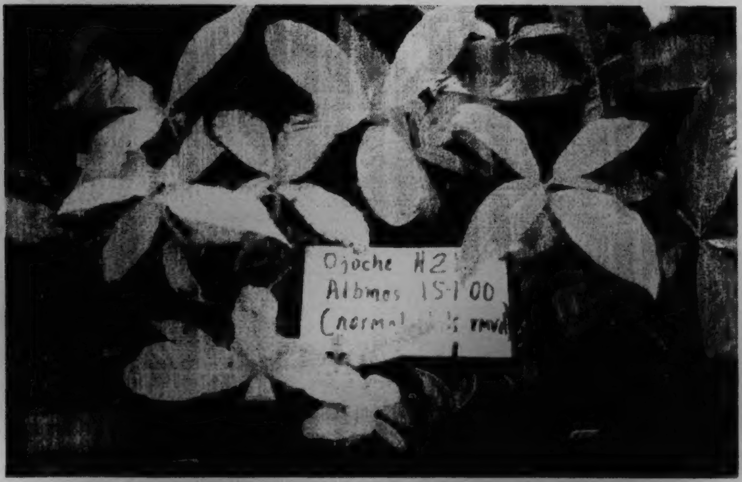
Ojoche #2
Albinos 15-1-00
(normal
rmvd

Figure 5-22. Albino progeny of *Anacardium excelsum* tree 21, El Ojoche population, northwestern Costa Rica

Chapter 6

INHERITANCE, LINKAGE AND NEUTRALITY OF ALLOZYMES OF THE NEOTROPICAL TREE *PLUMERIA RUBRA* L. (APOCYNACEAE)

INTRODUCTION

Plumeria rubra L. (calichuche, flor blanca, frangipani, sacuanjoche) is a small to medium sized deciduous tree. It occurs naturally from Mexico to Panama (Standley and Williams, 1969; Woodson, 1938), where it is found principally on sea-cliffs, limestone outcrops, and canyon sides within seasonally dry zones (Haber, 1984; Pittier, 1978). The hermaphroditic flowers are pollinated by hawkmoths (Haber and Frankie, 1989). The winged seeds appear to be selected primarily for wind dispersal, although, as trees often overhang watercourses, dispersal in streamflow also seems inevitable.

The natural range of *P. rubra* includes much of the Pacific watershed of Central America, where cattle-raising and agriculture have replaced all but approximately 8 per cent of the original dry forest vegetation (Janzen, 1986). Outside protected areas, a large proportion of the remaining forest appears to be concentrated in linear riparian remnants of various types and degrees of degradation (see Chapter Three). The population genetics of the constituent species of these strips, such as *P. rubra*, are of interest for two principal reasons. Firstly, the 'genetic health' of such populations partly determines their ecological viability. Secondly, information on the population genetics of such species may generate insights into conservation genetics of other taxa with similar life histories and similarly patchy or fragmented distributions.

The suitability of allozymes as genetic markers for population genetics studies is partly due to their codominant inheritance. However, as various factors may cause apparent or real departures from codominance (Gillet and Hattemer, 1989; Strauss and Conkle, 1986), it should be established rather than simply assumed. In the present article, enzyme polymorphism and its genetic basis in five enzyme systems of *P. rubra* are described. Linkage relationships and neutrality of these enzymes are also reported, as these characteristics affect to some degree their usefulness in population genetics studies, e.g. Ritland and Jain's (1981) algorithm for outcrossing rate estimation assumes that loci are independent and selectively neutral between gametic union and point of census. The use of the selected markers in the characterization of the impact of forest fragmentation on seven *P. rubra* populations is described separately (see Chapter Seven).

MATERIALS AND METHODS

Field and laboratory procedures

In February to April of 1997, 1998 and 1999 open-pollinated seeds were collected from individual trees in 7 forest remnants located in the *canton* of Cañas, Guanacaste Province, Costa Rica (Table 7-2). Ripe capsules were collected directly from the trees using a pruning pole and attached basket. Seeds were extracted manually and stored until needed.

There appear to be no published enzyme extraction or electrophoresis protocols for *P. rubra.* For this reason, combinations of different extraction buffers, electrophoresis buffer systems and enzymes were screened in order to identify potentially useful markers. The systems reported (aspartate aminotransferase (AAT, E.C. 2.6.1.1), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), glucose-phosphate isomerase (PGI, E.C. 5.3.1.9), phosphoglucomutase (PGM, E.C. 5.4.2.2) and phosphogluconate dehydrogenase, (PGD, E.C. 1.1.1.43)) were selected based on their clarity of resolution or consistency with expected quaternary structure. Other enzyme systems were rejected for various reasons: because staining resolution or intensity was unsatisfactory on all tested buffer combinations (glutamic dehydrogenase, glucose dehydrogenase, isocitric dehydrogenase), because acceptable resolution or intensity was only inconsistently achieved (adenylate kinase, esterase, leucine aminopeptidase, malic enzyme, shikimic kinase), because of interpretation difficulties (malate dehydrogenase) or because polymorphy was not detected (diaphorase, meniadone reductionase).

Extracts of the five enzymes were prepared by crushing recently emerged radicles in Liengsiri *et al.*'s (1990) extraction buffer #9. The homogenate was absorbed to Whatman #3 filter paper wicks, and stored at -80°C until needed. Horizontal starch (Connaught Laboratories, Ontario) gel electrophoreses were carried out using pH7.0 histidine-tris (Pitel and Cheliak, 1984)) (ADH, PGM, PGD) and pH8.1 lithium borate / tris citrate (Ridgeway *et al.*, 1970) (AAT, PGI) buffer systems. Allozymes were visualized using staining protocols from Liengsiri *et al.* (1990) and Wendel and Weeden (1990). Laboratory procedures are described in detail elsewhere (see Appendix One). The isozymatically invariable *Pinus resinosa* Ait. (Fowler and Morris, 1977) was used as a control. Loci were named in ascending numerical order according to their mobility, whereas alleles of each locus were assigned alphabetic codes, 'A' representing the most common allele.

Inheritance

Segregation analysis followed the methodology outlined by Gillet and Hattemer (1989). The following specific null hypotheses, both corresponding to codominant allelic action, were tested here on progeny arrays of putatively heterozygous mother-trees: (i) for maternal genotype XY (X≠Y), $n_{XY} = n_{XX} + n_{YY}$; where n=numbers of progeny; (ii) for maternal genotype XY, $n_{XZ} = n_{YZ}$, (Z≠X, Y) (AAT, PGM). The binomial probabilities of observed ratios were calculated. Subsequently, the significance of departure from 1:1 of individual arrays was examined using the Dunn-Šidák method of sequential Bonferroni testing (Sokal and Rohlf, 1995). Under this procedure, the ascendingly ranked probabilities are compared sequentially to a steadily increasing critical value $1-(1-\alpha)^{1/n}$, where α is the chosen probability value (0.05 in this case) and n is the number of arrays not already tested; testing continues until the first nonsignificant array is detected. The significance of departure from 1:1 of the pooled segregation ratios for each enzyme was tested using the *G*-test with Williams's adjustment (Sokal and Rohlf, 1995).

Formally, Gillet and Hattemer's methodology requires independent parental genotyping, that is, use of parental germplasm. When parental material is unavailable, as in the present case, putatively heterozygous mother-trees must first be identified on the basis of the presence of two homozygote types in their progeny. However, this means of identifying heterozygous parents for itself implicitly assumes codominant inheritance, because only under codominant inheritance can all homozygotes be distinguished from heterozygotes. Therefore, the strongest formal conclusion that can be made is that the segregation ratios of the observed phenotypes are consistent with their proposed genetic interpretations. However, when phenotypes are consistent with the quaternary structure of the enzyme in question, and particularly when apparent heterozygotes are detected, the degree of confidence attached to as assertion of codominant inheritance on the basis of the progeny segregation does not appear to be critically less than when parental material is used.

Linkage disquilibrium

As neither haploid material nor progeny-test data were available, linkage disequilbirium coefficients based on frequencies of coupling and repulsion heterozygotes were not estimable. Therefore, values of Burrows's composite linkage disequilibrium coefficient Δ_{ij} and the corresponding correlation coefficient (Weir, 1979) werre estimated for each of the seven populations. Significant values of Δ_{ij} imply that there are differential frequencies of coupling and repulsion heterozygotes and/or non-random union of gametes (Roberds and Brotschol, 1985). Ohta's (1982) multiple population linkage disequilibrium (D) coefficients were also estimated. Ohta's partitioning of the variance of linkage disequilibrium is useful in elucidating the causes of observed disequilibria: when D_{IS}^2 (the variance of disequilibrium within a population) is less than D_{ST}^2 (the variance of correlation between different gametes of one subpopulation relative to that of the total population) and D'_{IS}^2 (variance of within gamete correlation in a subpopulation relative to the total population) is greater than D'_{ST}^2 (variance of disequilibrium of the total population), then population subdivision (i.e. genetic drift) rather than epistatic selection is likely to be the main cause of linkage disequilibria (Kremer and Zanetto, 1997; Ohta, 1982). For estimation of both Burrows's and Ohta's coefficients, all alleles except the most common were pooled to a synthetic allele (Kremer and Zanetto, 1997). Missing values were eliminated from the data set as these add no information on non-gametic or gametic correlations in allele frequencies. Data from all collection years were pooled. POPGENE (Yeh and Boyle, 1997) was used for estimation of all the above disequilibrium parameters.

Neutrality

POPGENE was also used to test for selective neutrality. It uses Stewart's algorithm for the Ewens-Watterson neutrality test, as detailed in Fuerst et al. (1977) and Manly (1985). One thousand iterations were employed for generation of simulated distributions of expected values of the *F* statistic, *i.e.* the sum of squared allele frequencies under the null hypothesis, under which the configuration of alleles corresponds to the equilibrium values for the infinite alleles mutation model (Hartl and Clark, 1989). Neutrality was tested by population and for pooled data.

RESULTS

Inheritance

Aspartate aminotransferase

There was one clearly resolved locus, with three putative alleles. The banding patterns were those expected for a dimeric enzyme, although putative BB, BC, and CC genotypes frequently showed indistinct banding at the A locus (Figure 6-1). On some gels the heterodimer band of the putative AC genotype was slightly anodal of the expected intermediate position (Figure 6-2). None of the pooled segregation ratios of the progeny of putatively heterozygous (AB or AC) mother-trees was significantly different from the 1:1 expectation (Table 6-1). The lowest probability associated with individual progeny arrays, i.e. p=0.07 (family 4014) (Table 6-2) is greater than the corresponding Dunn-Šidák critical value of $1\text{-}(0.95)^{.037}$= 0.002 (n=27), *i.e.* not significantly different from expectations under the null hypothesis.

Alcohol dehydrogenase

Three variable loci were detected. The most cathodal locus was too poorly and inconsistently resolved to permit interpretation. The most anodal locus (ADH*3*) showed the expected (May, 1998) dimeric banding pattern. Two putative alleles were detected (Figure 6-3). Segregation of the intermediate-mobility locus (ADH*2*) coincided completely with that of ADH*3*, but its banding pattern was characteristic of a monomeric enzyme (Figure 6-3). The pooled segregation ratios were not significantly different from expectations (Table 6-1). The lowest probability associated with individual progeny arrays, i.e. p=0.04 (family 1007) (Table 6-3) is greater than the corresponding Dunn-Šidák critical value of $1\text{-}(0.9)^{.023}$= 0.001 (n=44), *i.e.* not significantly different from expectations under the null hypothesis.

Glucose-phosphate isomerase

The PGI zymograms were generally complex. However, the most simple of them reveal four zones of activity (Figure 6-4). The most anodal of these was too poorly resolved to permit scoring, whereas the complexity of the zymograms precluded genetic interpretation of the two most cathodal loci.

PGI*3* appeared to present banding patterns consistent with those expected for a dimeric enzyme with three alleles (Figures 6-5 and 6-6). However, the putative allele PGI*3*-B occurs within the apparent range of mobility of the putative locus PGI*2*. Therefore, PGI*3*-B and its possible combinations with allele PGI*3*-A could be confused with polymorphisms correctly attributable to locus PGI*2*. For this reason, only segregation ratios of apparent AC mothers werre assessed (putative allele PGI*3*-C occurs anodal of the most common PGI3 allele, and there is therefore little risk of confusion with alleles of loci *1* and *2*), (Figures 6-6 and 6-7). The pooled segregation ratio did not depart from the expectation under codominant inheritance (Table 6-1). None of the individual arrays departed significantly from expectations (Table 6-4).

Phosphoglucomutase (PGM; EC 5.4.2.2)

PGM*1* shows the monomeric banding pattern characteristic of this enzyme system (May, 1998), with three alleles (Figures 6-8, 6-9). None of the pooled PGM*1* segregation ratios showed departures from expectations under codominance (Table 6-1). None of the individual progeny arrays showed nominally significant departures from the 1:1 expectation (Table 6-5). Allele PGM*1*-B showed the same relative migration as the least common putative allele of the anodal PGM*2* locus. Potentially ambiguous cases were scored based on band intensity and unambiguous occurrence of the putative alleles elsewhere in the progeny array in question. Resolution of PGM*2* was inconsistent and did not permit genetic interpretation, although on the clearest gels (e.g. Figure 6-8) a polymorphic, monomeric banding pattern was evident.

Phosphogluconate dehydrogenase (PGD, EC 1.1.1.43)

Two single-banded phenotypes, i.e. putative homozygotes, and a three-banded putative heterozygote, consistent with expectations for this typically dimeric locus (May, 1998), were observed. However, genetic interpretation was complicated by the occurrence of two-banded phenotypes (Figures 6-10, 6-11). These consisted of one band of the same mobility as the heterodimer band, and one band of similar intensity at one of the two putative homodimer / homozygote positions. Initial segregation analysis without these phenotypes revealed a consistent homozygote excess in progeny of heterozygous mother-trees. It was therefore hypothesized that the double-banded phenotypes represent additional AB heterozygotes. The pooled segregation ratio calculated on [illegible]s basis showed a non-significant heterozygote excess (Table 6-1). The lowest probability associated with individual progeny arrays, i.e. p=0.01

(family Pachanga 7) (Table 6-6) is greater than the corresponding Dunn-Šidák critical value of $1\text{-}(0.9)^{.0192}= 0.001$ (n=52), *i.e.* not significantly different from expectations under the null hypothesis.

Linkage disequilibrium

There were significant ($p<0.05$) linkage disequilibria in three of the 70 permutations of 7 populations and 10 locus-pairs (Table 6-7). Three of the populations each showed one significant disequilibrium, but not for the same pair of loci. Of the 10 possible pairwise locus combinations, three evinced significant linkage disequilibria. All estimates of correlations between loci were $< |0.13|$. For all pairwise combinations, D_{IS}^2 was less than D_{ST}^2 and $D'_{IS}{}^2$ was greater than $D'_{ST}{}^2$ (Table 6-8), suggesting that genetic drift rather than epistatic selection is responsible for the the observed disequilibria.

Selective neutrality

Observed values of AAT, PGI and PGM were within the 95 per cent confidence limits for selectively neutral loci. In the case of ADH and PGD, observed values of F were generally close to, and in one case (ADH, Corobicí) less than, the lower 95 per cent confidence limits (Table 6-9), i.e. suggesting consistently more even allele frequencies than expected under neutrality.

DISCUSSION

Inheritance

The segregation ratios of these loci are consistent with the hypothesis of codominant inheritance, suggesting that, in these populations of this species, factors that may lead to departure or apparent departure from codominance, such as meiotic drive, null allelic action, and pre- or immediate postzygotic selection (Strauss and Conkle, 1986; Xie *et al.*, 1991) are not important for these loci.

With the partial exception of PGD*1*, banding patterns accorded with expected quaternary structure. As the foregoing interpretation of the double-banded phenotypes in PDG*1* is itself based partly on observed segregation ratios, the non-significance of departure from expected segregation ratios in this locus represents less conclusive evidence of codominance than in the other loci. However, alternative explanations appear to be untenable. Interpretation of these phenotypes as double-banded homozygotes would imply a large excess of homozygotes within progeny of heterozygotes and a similar excess of homozygosity in the progeny as a whole, whilst, by contrast, the maternal genotypes would be inferred as largely heterozygous, due to the higher prevalence under this interpretation of arrays with both putative homozygotes. The present interpretation implies partial null action of these alleles when in the heterozygous condition. The similar staining intensity of the two bands suggests that, whilst a heterodimer of intermediate polarity is formed, the active component is contributed by only one of the alleles, i.e. the one inactive in the homodimer state is also inactive in the heterodimer. The occurrence of both double-banders and 'typical' heterozygotes within the same families suggests that this polymorphism may be artefactual, rather than caused by defective forms of the alleles themselves.

Linkage disequilibrium

With 70 permutations of population and locus-pair, chance (i.e. type two error) would be expected to result in three to four estimates with associated probabilities of ≤ 0.05. The probability associated with one of the estimates (ADH/PGI, Palmira population) approaches this level; chance would appear to be the most parsimonious explanation for this observed disequilibrium.

The low probability values associated with the other disequilibria make the above explanation less likely, and suggest some linkage disequilbria in this tropical angiosperm, a result consistent with findings in temperate broadleaves (Granger, 1996; Huang *et al.*, 1996; Roberds and Brostchol, 1985; Zanetto *et al.*, 1996) and temperate and boreal conifer species (Cheliak and Pitel, 1985; Strauss and Conkle, 1986; Xie *et al.*, 1991; Yang and Yeh, 1993; Yeh *et al.*, 1994). There is insufficient information to distinguish between structural (i.e. location on the same chromosome) and non-structural causes of the observed linkage disequilibria. As gametic chromosome number of *P. rubra* appears to be n=18 (Bawa, 1973; van der Laan and Arends, 1985), and assuming that the loci on different chromosomes have equal chances of being selected for study, then the probability of any two of the five loci belonging to the same linkage group is $p = 1-[(18)(17)(16)(15)(14) \,/\, 18^5] = 0.45$, indicating that, *a priori*, it is moderately likely that physical linkage is responsible for one of the observed disequilibria.

Linkage disequilibrium of either structural or non-structural origin can be caused either by epistatic selection or genetic drift. The values of Ohta's coefficients, together with the lack of consistency in the direction (sign) of the estimated significant and non-significant disequilibria (Table 6-8), suggest that selective forces are not responsible for the observed disequilibria. Non-directional forces such as founder effects, population subdivision and parental sampling effects (Yeh and Morgan, 1987) all represent possible causal factors. For many applications, e.g. mating systems studies, the causes of observed disequilibria are of less interest than their magnitude. Close linkage, structural or otherwise, leads to underestimates of outcrossing rates (Brown *et al.*, 1985; Yeh and Morgan, 1987). In the present case, correlations between loci are relatively weak (i.e. $< |0.13|$) and found only in three populations. As such, it is unlikely that they will appreciable affect estimates of mating system and other parameters.

Neutrality

The results of the Ewens-Watterson test suggest non-neutrality in both ADH and PGD. Observed '*F*' less than or equal to the lower confidence limit suggests greater heterozygosity than expected under the infinite (neutral) alleles model, i.e. heterozygote superiority. However, the lack of departure from expected segregation ratios under codominance suggest that the selective forces responsible for maintaining the higher than expected levels of heterozygosity are absent (ADH) or relatively weak (PGD) at the sampled life-history stage (i.e. young progeny). It follows that the non-neutrality detected here is unlikely to influence parameter estimates, including mating parameters, based on young progeny material.

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Table 6-1. Segregation ratios in pooled progeny arrays of mother-trees putatively heterozygous at five allozyme loci of *Plumeria rubra.*

Enzyme	Maternal genotype	Classes for *G*-test	Observed numbers per class	n_{total}	G_{adj}[1]
*AAT*1	AB	AA+BB	231	441	0.99
		AB	210		
	AB	AC	9	21	0.42
		BC	12		
	AC	AA+CC	76	138	1.42
		AC	62		
	AC	AB	15	28	0.14
		BC	13		
	BC	BB+CC	3	7	0.13
		BC	4		
	BC	AB	15	33	0.27
		BC	18		
*ADH*3	AB	AA+BB	774	1546	0.003
		AB	772		
PGI*3*	AC	AA+CC	78	140	1.83
		AC	62		
PGM*1*	AB	AA+BB	43	86	0.00
		AB	43		
	AC	AA+CC	56	115	0.08
		AC	59		
	AB	AC	6	9	0.97
		BC	3		
	AC	AC	2	2	n.a.
		BC	0		
PGD1	AB	AA+BB	834	0.76	0.76
		AB	870		

[1] G-statistic with Williams's adjustment (Sokal and Rohlf, 1995); probability of all values is >0.05 (5% critical value of G-test is 3.84)

Table 6-2. Segregation ratios and associated probabilities in progeny of mother trees of *Plumeria rubra* putatively heterozygous for the AAT*1* locus.

Population, maternal genotypes	Family	Observed numbers per class				n_{total}	n_{exp}	p[1]
AB mothers		n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}			
Corobicí	1001	14	1	15	15	30	15	1.00
Corobicí	1017	15	10	25	28	53	26.5	0.78
Corobicí	1018	2	8	10	10	20	10	1.00
La Pachanga	8	2	1	3	2	5	2.5	1.00
Magdalena	4003	13	6	19	22	41	20.5	0.76
Magdalena	4009	16	9	25	19	44	22	0.45
Magdalena	4010	17	4	21	19	40	20	0.87
Magdalena	4014	17	4	21	10	31	15.5	0.07
Magdalena	4016	22	6	28	30	58	29	0.90
Palmira	1-300	11	2	13	9	22	11	0.52
Palmira	1-307	5	4	9	14	23	11.5	0.40
Palmira	7	8	5	13	7	20	10	0.26
San Isidro	2021	1	8	9	7	16	8	0.80
San Isidro	2027	19	1	20	18	38	19	0.87
AC mothers		n_{AA}	n_{CC}	$n_{AA}+n_{CC}$	n_{AC}			
Palmira	1-200	16	5	21	12	33	16.5	0.16
Praderas	2048	2	21	23	22	45	22.5	1.00
Praderas	2049	3	7	10	7	17	8.5	0.63
Praderas	2040	9	2	11	10	21	10.5	1.00
Praderas	2047	1	10	11	11	22	11	1.00
AB mothers		n_{AC}	n_{BC}					
Corobicí	1018	3	5			8	4	0.73
La Pachanga	8	0	1			1	0.5	1.00
Magdalena	4009	3	0			3	1.5	0.25
Magdalena	4014	2	2			4	2	1.00
San Isidro	2027	1	4			5	2.5	0.38
AC mothers		n_{AB}	n_{CB}					
Palmira	1-200	13	10			23	11.5	0.68
Praderas	2040	2	1			3	1.5	1.00
Praderas	2047	0	2			2	1.0	0.5

[1] two-tailed exact binomial cumulative probability under the null hypothesis of observed numbers of progeny in either class

Table 6-3. Segregation ratios and associated probabilities in progeny of mother trees of *Plumeria rubra* putatively heterozygous for ADH*3* alleles A and B.

Population	Family	Observed numbers per class				n_{total}	n_{exp}	p^1
		n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}			
Corobicí	1000	1	17	18	21	39	19.5	0.75
Corobicí	1003	15	14	29	19	48	24	0.19
Corobicí	1005	13	12	25	20	45	22.5	0.55
Corobicí	1006	12	17	29	41	70	35	0.19
Corobicí	1007	17	10	27	13	40	20	0.04
Corobicí	1009	7	11	18	25	43	21.5	0.36
Corobicí	1010	7	11	18	16	34	17	0.86
Corobicí	1011	9	16	25	23	48	24	0.88
Corobicí	1012	5	22	27	25	52	26	0.89
Corobicí	1017	17	16	33	30	63	31.5	0.80
La Pachanga	C4	6	13	19	13	32	16	0.38
La Pachanga	C7	3	8	11	10	21	10.5	1.00
La Pachanga	5	2	5	7	1	8	4	0.07
La Pachanga	6	7	9	16	12	28	14	0.57
La Pachanga	7	3	4	7	17	24	12	0.06
La Pachanga	3	1	4	5	6	11	5.5	1.00
Magdalena	4000	8	17	25	16	41	20.5	0.21
Magdalena	4005	3	6	9	9	18	9	1.00
Magdalena	4007	7	13	20	29	49	24.5	0.25
Magdalena	4008	3	20	23	22	45	22.5	1.0
Magdalena	4009	8	17	25	28	53	26.5	0.78
Magdalena	4011	2	26	28	40	68	34	0.18
Magdalena	4014	2	15	17	18	35	17.5	1.00
Magdalena	4018	2	8	10	7	17	8.5	0.63
Palmira	1-300	6	1	7	12	19	9.5	0.36
Palmira	1-306	11	1	12	12	24	12	1.00
Palmira	1-307	4	7	11	11	22	11	1.00
Praderas	2037	12	6	18	22	40	20	0.64
Praderas	2040	2	9	11	13	24	12	0.84
Praderas	2042	8	17	25	34	59	29.5	0.30
Praderas	2043	5	8	13	7	20	10	0.26
Praderas	2047	10	3	13	12	25	12.5	1.00
Praderas	2048	7	21	28	17	45	22.5	0.14
Praderas	2054	3	1	4	5	9	4.5	1.00
Praderas	2055	15	13	28	25	53	26.5	0.78

[1] two-tailed exact binomial cumulative probability under the null hypothesis of observed numbers of progeny in either class

Table 6-3. Segregation ratios and associated probabilities in progeny of mother trees of *Plumeria rubra* putatively heterozygous for ADH*3* alleles A and B (continued)

Population	Family	Observed numbers per class				n_{total}	n_{exp}	p^1
		n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}			
San Isidro	2010	6	12	18	24	42	21	0.44
San Isidro	2011	15	10	25	22	47	23.5	0.77
San Isidro	2017	4	5	9	9	18	9	1.00
San Isidro	2018	11	6	17	22	39	19.5	0.52
San Isidro	2022	9	4	13	16	29	14.5	0.71
San Isidro	2026	8	9	17	15	32	16	0.86
San Isidro	2030	7	6	13	7	20	10	0.26
San Isidro	2034	10	9	19	28	47	23.5	0.24

[1] two-tailed exact binomial cumulative probability under the null hypothesis of observed numbers of progeny in either class

Table 6-4. Segregation ratios and associated probabilities in progeny of mother trees of *Plumeria rubra* putatively heterozygous for locus PGI*3*.

Population, maternal genotypes	Family	Observed numbers per class				n_{total}	n_{exp}	p^1
AC mothers		n_{AA}	n_{CC}	$n_{AA}+n_{CC}$	n_{AC}			
Corobicí	1018	13	3	16	10	26	13	0.33
La Pachanga	8	2	1	3	1	4	2	0.63
Magdalena	4016	21	1	22	24	46	23	0.88
Magdalena	4017	15	1	16	9	25	12.5	0.23
Quebrada Palmira	1-307	1	9	10	8	18	9	0.81
Quebrada Palmira	qp12	9	2	11	10	21	10.5	1.00

[1] two-tailed exact binomial cumulative probability under the null hypothesis of observed numbers of progeny in either class

Table 6-5. Segregation ratios and associated probabilities in progeny of mother trees of *Plumeria rubra* putatively heterozygous for the PGM*1* locus

Population, maternal genotypes	Family	Observed numbers per class				n_{total}	n_{exp}	p^1
AB mothers								
		n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}			
Corobicí	1004	25	1	26	28	54	27	0.66
San Isidro	2022	10	6	16	15	31	15.5	1.00
AC mothers								
		n_{AA}	n_{CC}	$n_{AA}+n_{CC}$	n_{AC}			
Corobicí	1006	23	2	25	30	55	27.5	0.59
	1010	22	1	23	22	45	22.5	1.00
Q. Palmira	1	5	1	6	6	12	6	1.00
		n_{AC}	n_{BC}					
AB mothers								
1004		6	2			8	4	0.29
AC mothers		n_{AB}	n_{BC}					
Q. Palmira	1	2	0			2	1	0.50

[1] two-tailed exact binomial cumulative probability under the null hypothesis of observed numbers of progeny in either class

Table 6-6. Segregation ratios and associated probabilities in progeny of mother trees of *Plumeria rubra* putatively heterozygous for locus PGD*1*.

Population	Family	Observed numbers per class				n_{total}	n_{exp}	p^1
		n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}			
Qbrda. Palmira	100	31	1	32	17	49	24.5	0.04
	300	2	2	4	12	16	8	0.08
	309	7	1	8	11	19	9.5	0.65
	10	5	2	7	4	11	5.5	0.55
	12	5	4	9	11	20	10	0.82
	2	6	1	7	16	23	11.5	0.09
	3	3	2	5	5	10	5	1.00
	7	2	6	8	9	17	8.5	1.00
	9	2	5	7	5	12	6	0.77
Corobicí	1000	12	5	17	16	33	16.5	1.00
	1001	14	1	15	31	46	23	0.03
	1004	24	3	27	21	48	24	0.47
	1005	11	7	18	20	38	19	0.87
	1006	13	13	26	33	59	29.5	0.43
	1008	24	5	29	23	52	26	0.49
	1010	11	7	18	20	38	19	0.87
	1011	6	19	25	15	40	20	0.15
	1012	3	11	14	21	35	17.5	0.31
	1013	8	6	14	11	25	12.5	0.69
	1017	22	6	28	31	59	29.5	0.79
	1018	6	14	20	17	37	18.5	0.74
	1021	14	6	20	28	48	24	0.31
San Isidro	2010	2	17	19	14	33	16.5	0.49
	2011	13	9	22	20	42	21	0.88
	2013	1	2	3	6	9	4.5	0.51
	2018	13	6	19	7	26	13	0.03
	2021	5	4	7	11	18	9	0.48
	2021	5	2	7	11	18	9	0.48
	2022	12	0	12	14	26	13	0.85
	2027	11	3	14	29	43	21.5	0.03
	2034	0	25	25	13	38	19	0.07
	2041	22	5	27	31	58	29	0.69
	2047	8	6	14	8	22	11	0.29
	2051	11	5	16	20	36	18	0.62

[1] two-tailed exact binomial cumulative probability under the null hypothesis of observed numbers of progeny in either class

Table 6-6. Segregation ratios and associated probabilities in progeny of mother trees of *Plumeria rubra* putatively heterozygous for locus PGD (continued)

Population	Family	Observed number per class				n_{total}	n_{exp}	p[1]
		n_{AA}	n_{BB}	$n_{AA}+n_{BB}$	n_{AB}			
Magdalena	4000	7	2	9	10	19	9.5	1.00
	4003	11	6	17	17	34	17	1.00
	4007	15	4	19	24	43	21.5	0.54
	4008	18	2	20	25	45	22.5	0.55
	4009	12	10	22	29	51	25.5	0.40
	4010	19	2	21	15	36	18	0.41
	4013	21	6	27	19	46	23	0.30
	4016	6	8	14	16	30	15	0.86
	4019	16	4	20	24	44	22	0.65
	4020	12	4	16	20	36	18	0.62
Pachanga	CS7	5	9	14	7	21	10.5	0.19
	P1	10	0	10	10	20	10	1.00
	P2	7	7	14	9	23	11.5	0.40
	P5	3	2	5	4	9	4.5	1.00
	P6	5	2	7	21	28	14	0.01
	p7	3	10	13	7	20	10	0.26
Sandillal	r00	14	7	21	25	46	23	0.66
	r02	19	3	22	27	49	24.5	0.57

[1] two-tailed exact binomial cumulative probability under the null hypothesis of observed numbers of progeny in either class

Table 6-7. Significant estimates of Burrows's composite linkage disequilibrium coefficient (Δ_{ij}), correlation coefficient (r) and significance (p) of associated chi-squared test in populations of 7 populations of *Plumeria rubra* in Guanacaste province, Costa Rica.

Locus / alleles[1]	Population	Δ_{ij}	r	p	n[2]	+N:-N[3]
AAT-A/PGM-A	Corobicí	-0.0113	0.0171	.0165	720	5/2
ADH-A/PGI-A	Palmira	-0.0213	-0.1139	0.0391	328	3/4
ADH-A/PGM-A	San Isidro	0.0233	0.124	0.007	460	3/4

[1] All alleles except the most frequent were pooled to one synthetic allele; [2] number of individual genotypes observed; [3] *i.e.* numbers of positive and negative correlations, irrespective of significance, over all 7 populations.

Table 6-8. Estimates of Ohta's multiple population linkage disequilibrium coefficients for 7 populations of *Plumeria rubra* in northwestern Costa Rica.

Loci	D_{IT}^2	D_{IS}^2	$D'_{IS}{}^2$	D_{ST}^2	$D'_{ST}{}^2$
AAT/ADH	.06148	.00036	.059083	.06132	.00166
AAT/PGI	.02186	.00011	.02183	.02205	.00002
AAT/PGM	.02695	.00065	.02693	.02478	.00001
AAT/PGD	.02869	.00050	.02855	.02644	.00014
ADH/PGI	.04148	.00025	.04145	.04304	.00003
ADH/PGM	.04945	.00052	.04943	.04559	.00002
ADH/PGD	.04005	.00067	.03953	.03879	.00052
PGI/PGM	.00802	.00001	.00800	.00813	.00003
PGI/PGD	.02059	.00009	.02042	.02039	.00017
PGM/PGD	.02215	.00026	.02202	.02091	.00013
Overall	**.03207**	**.00034**	**.03180**	**.03114**	**.00027**

Table 6-9. Results of Ewens-Watterson test for selective neutrality of five allozyme loci in 7 populations of *Plumeria rubra* in Guanacaste province, Costa Rica

Locus	Parameters	Population							
		Palmira	**Corobicí**	**San Isidro**	**Praderas**	**Magdalena**	**Pachanga**	**Sandillal**	**All**
AAT	**n**[1]	606	1528	852	806	1278	330	376	5776
	k[2]	3	3	3	3	3	3	2	3
	F_{obs}[3]	0.51	0.64	0.53	.54	.69	.60	.95	0.61
	95% limits[4]	0.40 -0.99	0.39-1.0	0.40 -0.99	.42 -.99	.40 -.99	.38-.98	.50-.99	0.44-.99
ADH	**n**[1]	670	1672	936	1000	1386	336	420	6420
	k[2]	2	2	2	2	2	2	2	2
	F_{obs}[3]	0.51	0.52	0.50	0.51	0.65	0.51	0.93	0.53
	95% limits[4]	0.50-1.0	0.50-1.0	0.51 – 1.0	0.51-1.0	0.51 – 1.0	0.50-0.99	0.50-1.0	0.50-.99
PGI	**n**[1]	714	1770	882	894	1378	338	430	6406
	k[2]	2	2	2	2	2	2	2	2
	F_{obs}[3]	0.80	0.92	0.89	0.98	0.88	0.98	0.78	0.90
	95% limits[4]	0.50-1.0	0.51-1.0	0.50-1.0	0.51-1.0	0.50-1.0	0.50-0.99	0.50-0.99	0.51-.99
PGD	**n**[1]	512	1410	788	694	1204	332	326	5234
	k[2]	2	2	2	2	2	2	2	2
	F_{obs}[3]	0.52	0.51	0.50	.63	0.58	0.51	0.57	0.53
	95% limits[4]	0.51-0.99	0.51-1.0	0.50-1.0	0.51-1.0	0.50-1.0	0.50-0.99	0.50-0.99	0.50-.99

[1]Sample size, i.e. twice the number of progeny sampled; [2]number of alleles in sample; [3]observed *F*; [4]95 per cent confidence limits of sampling distribution

Table 6-9. Results of Ewens-Watterson test for selective neutrality of five allozyme loci in 7 populations of *Plumeria rubra* in Guanacaste province, Costa Rica (continued)

Locus	**Parameters**	**Population**							
		Palmira	**Corobicí**	**San Isidro**	**Praderas**	**Magdalena**	**Pachanga**	**Sandillal**	**All**
PGM	**n**[1]	666	1676	944	996	1434	332	352	6400
	k[2]	3	3	3	3	3	2	2	3
	F_{obs}[3]	0.89	0.78	0.69	0.91	0.93	0.96	0.93	0.85
	95% limits[4]	0.40-0.99	0.42-1.0	0.40-0.99	0.40-0.99	0.39-1.0	0.50-0.99	0.50-0.99	0.44-.99

[1]Sample size, i.e. twice the number of progeny sampled;[2]number of alleles in sample[3]observed *F*;[4]95 per cent confidence limits of sampling distribution

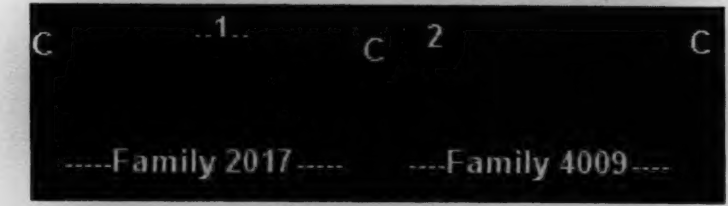
C
1
C
2
C
-----Family 2017-----
----Family 4009----

Figure 6-1. Aspartate aminotransferase zymogram of *Plumeria rubra*, showing (1) faint banding in A-locus (most cathodal) position in BC and BB genotypes (marked respectively *1* and *2*); *C* indicates the control (*Pinus resinosa*).

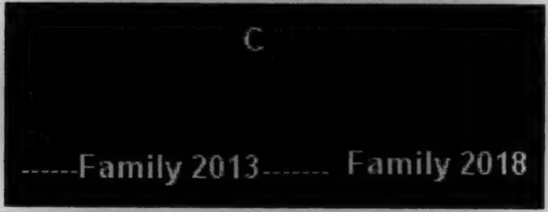
C
------Family 2013------
Family 2018

Figure 6-2. Aspartate aminotransferase zymogram of *Plumeria rubra*, showing asymmetry of AC heterodimers in two families. 'C' indicates the control (*Pinus resinosa*).

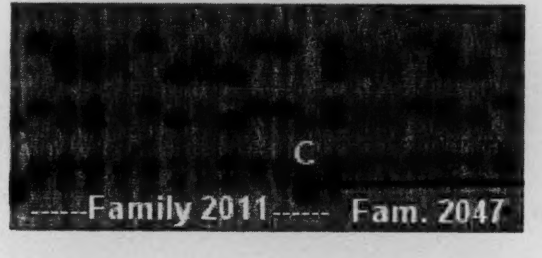
C
------Family 2011------
Fam. 2047

Figure 6-3. Alcohol dehydrogenase zymogram of *Plumeria rubra*. ADH*2* (intermediate mobility) shows an apparently monomeric banding pattern and apparent close linkage with the dimeric, most anodal locus. 'C' indicates the control (*Pinus resinosa*).

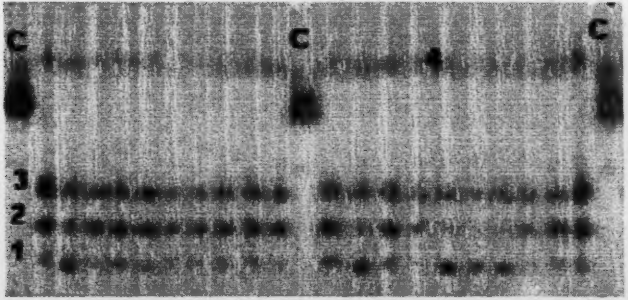

Figure 6-4. Example of less complex glucose-phosphate isomerase zymogram of *Plumeria rubra*, showing four zones of activity (putative loci, labelled 1 to 4). 'C' indicates the control (*Pinus resinosa*).

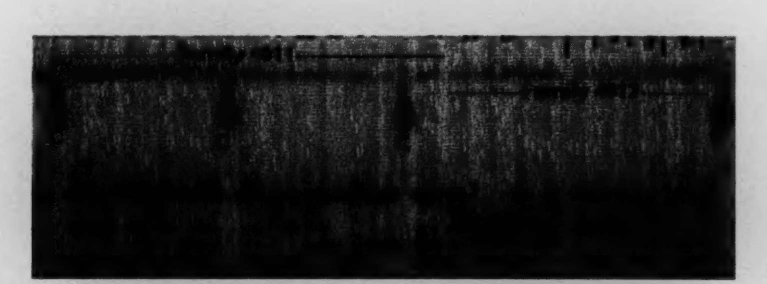

Figure 6-5. Glucose-phosphate isomerase zymogram of *Plumeria rubra*, showing putative genotypes of locus 3: 1=AA, 2=AB, 3=BB; C indicates control (*Pinus resinosa*).

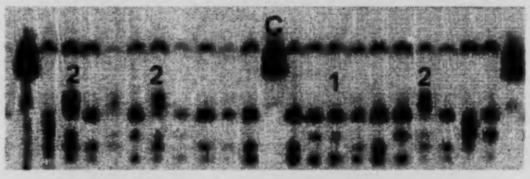

Figure 6-6. Glucose-phosphate isomerase zymogram of *Plumeria rubra*, showing putative genotypes of locus 3: 2=AC, 1=AA; C indicates control (*Pinus resinosa*); families 2026 (first lane), 2034 (final two lanes), 2027 (other lanes).

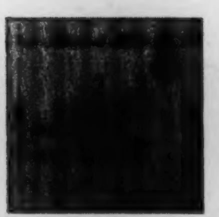

Figure 6-7. Glucose-phosphate isomerase zymogram of *Plumeria rubra*, showing putative genotypes of locus 3: 1=AA; 2=AC; 3=CC. C indicates control *(Pinus resinosa)*. Family QP12.

Figure 6-8. Phosphoglucomutase zymogram of *Plumeria rubra*, showing putative genotypes at locus PGM*1*: AA (marked 1), AB (marked 4).

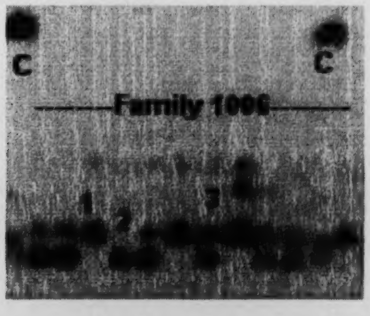

Figure 6-9. Phosphoglucomutase zymogram of *Plumeria rubra*, showing putative genotypes at locus PGM*1*: AA (marked 1), CC (marked 2, AC (marked 3).

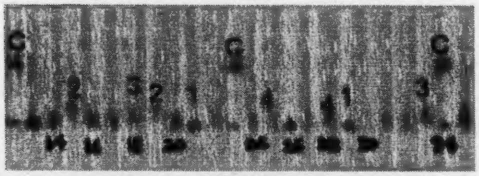

Figure 6-10. Phosphogluconate dehydrogenase zymogram of *Plumeria rubra*, showing putative genotypes: 1=AA, 2=BB, 3=AB, 4=double-banded phenotypes scored as AB (see text); 'C' indicates control (*Pinus resinosa*).

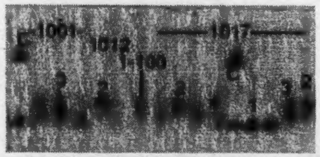

Figure 6-11. Phosphogluconate dehydrogenase zymogram of *Plumeria rubra*, showing putative genotypes: 1=AA, 2=BB, 3=AB, 4=double-banded phenotypes scored as AB (see text) (family 1004); 'C' indicates control (*Pinus resinosa*).

Chapter 7

THE EFFECTS OF FOREST FRAGMENTATION ON GENETICS AND REPRODUCTION OF THE TREE *PLUMERIA RUBRA* L. IN NORTHWESTERN COSTA RICA

INTRODUCTION

Tropical forests were destroyed at a rate of around 15.2 million ha year^{-1} in the decade 1990-2000 (FAO, 2001). However, deforestation is often not complete. Rather, in many cases, one or more tracts within the formerly continuous tree cover remain forested, and are converted by deforestation into fragments set in an unforested matrix. If biologically viable, such fragments may mitigate some negative consequences of deforestation. It follows that the biological implications, including genetic aspects, of the conversion of forest tracts to forest fragments are of considerable relevance to the management and conservation of forests and biodiversity.

The possible effects of forest fragmentation on genetic diversity are complex and interacting. The most immediate of these effects occurs at fragmentation, which, for species formerly present in deforested matrices, leads to reduction in population size. Depending on their size and allele frequencies, reduced populations may not contain all alleles formerly present, *i.e.* they may show founder effects (Meffe and Carroll, 1994; Yeh 2000). Continued low population size is expected to lead to further loss of variation due to random genetic drift (Hartl and Clark, 1989), a process which may be exacerbated by changed post-fragmentation environment. The latter has the potential to cause additional reductions in population size and higher variation in fertility (*e.g.* Aldrich and Hamrick, 1998; Kelly *et al.*, 2000), thereby reducing the ratio N_e/N (effective to census population sizes) (Nunney, 1993; Falconer, 1989). The effects of fragmentation on inter- and intra-population gene flow may exacerbate or mitigate such responses. For example, disturbance-mediated declines in density of tree populations may, due to decline in inter-tree pollinator movements (Karron *et al.*, 1995; Ghazoul *et al.* 1998), lead to increased geitonogamous selfing, which may also be caused by disturbance-mediated changes in pollinator assemblages (Aizen and Feinsinger, 1994). Increased selfing may have an immediate negative impact on fitness, *e.g.* by causing inbreeding depression (Gigord *et al.* 1998), and also increases susceptibility to drift by further reducing N_e (Yeh 2000). Maintenance of prefragmentation levels of gene flow may, with time, restore variation lost in founder events and may also prevent cumulative drift. Although the proportion of immigrant seed and pollen in a given fragment *x* may be lower than when the fragment was a tract in continuous forest, at the same time it may originate to a higher degree than previously from fragments located further from *x* than those extinct pollen and seed sources once present in the matrix. When genetic distance correlates with physical distance, such migrants may be more effective, because of their greater genetic divergence (Mills and Allendorf, 1996). Furthermore, as suggested above, fragmentation and concomitant disturbance may reduce the number of pollination events involving 'home' pollen, implying higher migration rate for a given amount of incoming pollen.

Clearly, the effect of fragmentation on genetic diversity is not easy to predict, particularly given the relatively limited empirical information available on responses of tree species to fragmentation (Chapter 2). In the present document, the effects of forest fragmentation on the reproduction and population genetics of *Plumeria rubra* (calichuche, flor blanca, frangipani, sacuanjoche) are reported. *P. rubra* is a small to medium sized deciduous tree native from Mexico to Panama (Standley and Williams, 1969; Woodson, 1938), where it is found principally on sea-cliffs, limestone outcrops, and canyon sides within seasonally dry zones (Haber, 1984; Pittier, 1978). Its pollination system is based on 'deceit'; the hawkmoth pollinator (Haber and Frankie, 1989) is attracted by the general conformity of the flowers to the 'hawkmoth syndrome', but there is no nectar reward (Haber, 1984). The latter author considers the absence of nectar in the case of *P. rubra* to be 'one of the great mysteries of pollination biology'. There is no published information on mating systems, although Haber (1984) remarks that the species's low ratio of fruit to flower production, given the close proximity of stigma and stamens, indicates self-incompatibility. The winged seeds appear to be selected primarily for wind dispersal, although dispersal in streamflow also seems inevitable where trees overhang watercourses. Individual trees can flower at two years age in favourable environments (Cornelius, J.P., personal observation). However, growth and development can be relatively slow in typical natural habitat. The largest trees in the present study zone are probably at least 30 years old.

In the study zone, *P. rubra* is found principally on river canyon-sides and rock outcrops. As a locally common species of naturally clumped distribution, information about the population genetics of *P. rubra* may generate insights of relevance to other taxa with similar life histories and similarly patchy or fragmented distributions. Additionally, as riparian forest appears to represent a large proportion of the remaining closed forest of this largely deforested zone, the 'genetic health' of *P. rubra* populations is linked to the conservation status of these habitats.

METHODS

Study zone and fragments

The study zone (Figure 7-1) is an area of approximately 100 km^2 located between 10°33' and 10°27' N, 85°05' and 85°10'W in the *canton* of Cañas, Guanacaste Province, northwestern Costa Rica. It is located in the northern sector of the wider study zone described in Chapter Three. Around 95% of the mean annual rainfall of 1693.4 mm (s=459.4) falls between May and November (San Luis, Cañas meteorological station, 1921-1978, MIRENEM, 1988). Altitude varies from 40-200m a.s.l; mean annual temperature at 95m a.s.l. is 27.5°C (Jiménez *et al.*, 1987). The dry season is characterized by strong (up to 90km hr^{-1}) northerly winds (Coen, 1983) and temperatures up to 37°C. The deforestation of the study zone occurred mostly during the last 80 years, as a consequence of three main factors: the replacement by exotic pasture species of the semi-open woodland ('sitios') formerly used for grazing, the conversion of closed forest to grassland, and the conversion of woodland to agriculture (see Chapter Three). Currently, the dominant land use is beef cattle ranching, and the study zone consists in the main part of pastureland dissected by the predominantly northeast-southwest orientated tributaries of the Tenorio River. Forest in the study zone is concentrated along these watercourses (Chapter Three). Non-riparian forest is essentially confined to young secondary (*e.g.* abandoned pastureland) or degraded primary remnants.

Within the zone, 7 populations were located using maps, aerial photographs and field exploration (Figure 7-1, Table 7-1). All populations except Pachanga and Sandillal are located in linear riparian forest. The Pachanga trees are drawn from two foci of *P. rubra* within an area of mixed pastureland, semi-abandoned pastureland, young secondary forest and canyon forest between the Magdalena and Corobicí Rivers and the hamlet of Correntadas. In this area, the species occurs as widely-spaced small groups and individual trees. The Sandillal population is located on a ridge-edge overlooking the River Santa Rosa.

The study populations are not drawn from wholly discrete forest fragments set in a treeless matrix. Rather, they represent components of a degraded dentritic forest network. The degree of discontinuity and attrition of forest cover within this network varies greatly, *i.e.* from zero in stretches of intact primary forest, through sectors reduced to narrow bands of forest on both sides, to stream reaches bordered by isolated trees, to complete absence of forest on one or both river banks. Trees (although only very rarely *P. rubra*) are also relatively common in the pastureland matrix (Chapter Three). The distribution of *P. rubra* in the study zone is characterized by the occurrence of population foci or 'swarms' in sites where the species has a strong competitive advantage, *i.e.* canyon-sides and rocky-banked river reaches. In general, such forest has been less affected by deforestation, agricultural encroachment and grazing damage than more accessible sites on deeper and more productive soils. Consequently, these processes have increased the degree of isolation of *P. rubra* populations within the study zone.

Field and laboratory procedures

Individual trees in each fragment were mapped and measured for dbh (except Praderas population). Neighbourhood density index (*NDI*) of each tree was calculated as:

$$NDI = \frac{\sum_{i=1}^{n}\left(t_{25} + 0.5t_{50} + 0.25t_{100}\right)_i}{n}$$

where $t_{25/50/100}$ = number of mature *P. rubra* trees within 0-25m, 25-50m, 50-100m of each sample tree. The index is a distance-weighted measure of the number of conspecifics within 100m of the tree in question, *i.e.* trees at 50-100m and 25-50m are given respectively one quarter and one half of the weighting of trees located within 25m. Population mean NDI and dbh were calculated (Table 7-1).

Seed collections were made from February to April of 1997 (all except Pachanga, Sandillal, Quebrada Palmira), 1998 (all except La Pachanga) and 1999 (all except San Isidro and Praderas). All trees with ripe fruit were collected in each case, except in those few cases where trees were inaccessible. Ripe capsules were collected directly from the trees using a pruning pole and attached basket. Seeds were extracted manually and stored until needed. Starch gel electrophoresis was carried out on enzyme extracts prepared from recently emerged radicles. Five codominant isozyme loci (Chapter 6) were assayed: aspartate aminotransferase (AAT, E.C. 2.6.1.1), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), glucose-phosphate isomerase (PGI, E.C. 5.3.1.9), phosphoglucomutase (PGM, E.C. 5.4.2.2), phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.43). Extraction and electrophoresis protocols are documented fully elsewhere (see Appendix One). These loci were selected from a wider group of 16 allozymes based on resolution, ease of interpretation and polymorphism (Chapter Six). ADH and PGD appear to be non-neutral, but segregation ratios indicate that selection is absent or weak between fertilization and the census age used (*i.e.* recent germinants) (Chapter Six). The sampling schedule is detailed in Table 7-2.

Specific research questions

A priori, there are several reasons to expect that *P. rubra* might tend towards higher subpopulation differentiation and lower within-population variation than many other tropical broadleaves. First, its preferred discontinuous habitat results in a naturally-aggregated or fragmented population distribution. The aggregated distribution of *P. rubra* should also be contrasted with such distributions that result from temporary niches, *e.g.* due to catastrophic disturbance. Preferred *P. rubra* habitat, *e.g.* exposed rock on canyon sides, is not temporary in nature and appears to favour long persistence of small populations in the same place for long periods of time. In the absence of migration, such populations will be subject to loss of variation due to genetic drift. Second, abiotically-dispersed species are in general expected to show greater population differentiation than species whose diaspores are dispersed by highly mobile birds and mammals (Loveless, 1992). Although the occurrence of isolated single trees of *P. rubra* seems to demonstrate the possibility of long-distance dispersal of its samaras, these are nevertheless likely to be less mobile than seeds dispersed by animal vectors, which may actively seek neighbouring populations. Furthermore, although the dry season winds coincide with the seed dispersal period of *P. rubra*, the species habitat is often substantially sheltered from these winds, which would also tend to favour within-river, *i.e.* broadly north-south, movement. Third, apparent population size tends to be relatively small in *P. rubra*, *i.e.* often in tens rather than hundreds. Genetic drift is more likely to be an important evolutionary factor in these circumstances. Fourth, the population shape of *Plumeria rubra* is characteristically linear. Under both the isolation-by-distance and stepping stone migration models, population differentiation is likely to be increased by such unidimensionality (Hartl and Clark, 1989; Wright, 1969; Kimura and Weiss, 1964).

Given these considerations, the present study addresses the following issues: first, does the species show any sign of reduced genetic variation and high spatial genetic structure? Second, how has *P. rubra* in the study zone responded to forest fragmentation and its concomitant effects?

Genetic variation and structure

Genetic parameters were estimated for maternal and progeny generations. As maternal material was unavailable, maternal genotypes were inferred using the most-likely-parent method (Brown and Allard, 1970), as programmed in Ritland's MLTR program (DOS version 1.1, Ritland, 1996). Initially, inferences were made on pooled (across collection years and capsules) arrays. However, as there is correlated mating within capsules of *P. rubra* (see results), pooling of capsular arrays within progenies could lead to information loss and consequent incorrect maternal genotype inferences. For example, the maternal parent of an array of 40 progeny consisting of equal numbers of XX and XY (X≠Y) could (depending on allele frequencies) be inferred as XY, whereas if the array was known to be made up two capsular arrays, one exclusively XX and the other exclusively XY, the probability of maternal heterozygosity would be remote (*i.e.* 0.5^{20}) (in this case the genotypic composition of the array would most likely be attributable to fertilization by two homozygous genotypes XX and YY). Accordingly, inferred maternal genotypes were cross-checked against their corresponding capsular arrays and reassigned maternal genotype (*i.e.*, heterozygote to homozygote) where appropriate (see Appenndix Two for details). In the case of arrays which include two homozygous types, this procedure is unnecessary, as maternal genotype is known.

For the maternal generation and each of the progeny cohorts, allele frequencies and allelic richness (*A*) (mean number of alleles per locus) were calculated. As all the sampled loci were polymorphic, *A* in this case is equivalent to *AP* (mean number of alleles per polymorphic locus) (Berg and Hamrick, 1997). Nei's expected heterozygosity ($H_e = 1 - \sum_i^n p^2_i$, where p_i=frequency of allele *i*) (Weir, 1996) was also estimated and averaged over all assayed loci. The fixation index F_{is} ($1-H_o/H_e$, where H_o=observed heterozygosity) was calculated as a measure of heterozygote deficit or excess with regard to HWE expectations. The null hypothesis of HWE within each population was tested using G-tests. All alleles but the most common were pooled to one synthetic class for both the significance testing (to maximize the number of observations per expected genotypic class) and calculation of F_{is}, (for consistency between the measure of disequilibrium and its test statistics). However, due to the low population numbers, in many cases the number of observations was still ≤5, usually considered as the threshold for uncritical application of the G-test (Sokal and Rohlf, 1995).

Homogeneity tests were carried out to test the null hypothesis of no population differentiation. As for the tests of Hardy-Weinberg equilibrium, and for the same reasons, the less common alleles were pooled to one synthetic allele. Wright's F-statistics (F_{is}, correlation between uniting gametes relative to the subpopulations; F_{it}, correlation between uniting gametes relative to the population as a whole; F_{st}, correlation between two gametes drawn randomly within subpopulations relative to that of two gametes drawn randomly from the population as a whole) were estimated as:

$$F_{IS} = \frac{H_S - H_I}{H_S},$$

$$F_{IT} = \frac{H_T - H_I}{H_T},$$

$$F_{ST} = \frac{H_T - H_S}{H_T},$$

where H_I = observed average heterozygosity of individuals within subpopulations; H_S=expected average heterozygosity; H_T= total expected heterozygosity (gene diversity) for population as a whole (pooled subpopulations) (Yeh, 2000).

Average historical gene-flow was estimated using the F_{st} method (*i.e.* $mN=0.25(1-F_{st})/F_{st}$), where *N*=effective population size and m = average migration rate) (Yeh, 2000). Pairwise estimates of Nei's unbiased genetic distance were also calculated and UPGMA dendrograms (Weir, 1996) based on these were estimated. Significance of the relationship between pairwise genetic distance and pairwise geographical distance between fragments was tested using the Mantel test (Sokal and Rohlf, 1995) as programmed in Mantel Version 2.0 (Liedloff 1999).

Except where indicated, the above analyses were carried out using the software package POPGENE (Yeh and Boyle, 1997).

Current gene flow

The Sandillal population, which was completely sampled (*i.e.* sample size = 4 = population size) is monomorphic at three loci (see results). In this case, gene flow is estimable as:

$$m = \frac{q_t}{\overline{q}},$$

where m=proportion of immigrant alleles, q_t=allele frequency in the progeny generation, $\overline{q}$ = mean allele frequency in the source population (Hamrick and Nason, 2000). As the results demonstrate correlation between genetic and geographic distance, the nearest population (*i.e.* River Magdalena, see Figure 7-1) was assumed to be the pollen source. Allele frequencies in the Magdalena population were based on average frequencies over the three collection years. These estimates may be biased by pollen immigration to the source, but they were preferred to inferred maternal genotypes or ovule allele frequencies (estimable with MLTR (Ritland, 1996)) both because they allow both for differences in male fertility and for pollen donors not included in the sample of mother-trees.

Mating system

The multilocus outcrossing rate (t_m), average single-locus outcrossing rate (t_s) correlations of outcrossing rate (r_t) and outcrossed paternity (r_p) were estimated by fragment using Ritland's MLTR programme (DOS Version 1.1) (Ritland, 1996), which employs Ritland and Jain's (1981) mixed mating system model. Within MLTR, the likelihood equations were solved using the EM method, and standard errors computed with 1000 bootstraps. Estimates of t less than

$1-2s_{\bar{x}}$ were considered to depart significantly from full outcrossing (Liengsiri *et al.*, 1998); analogous criteria were used for r_p, r_t , *i.e.* estimates greater than $2s_{\bar{x}}$ were considered to differ significantly from zero. MLTR was also used for estimation of individual outcrossing rates and pollen allele frequencies. For these estimations, progeny arrays were pooled across collection years in order to maximize sample size. The significance of between-fragment differences in mean individual-tree outcrossing rate was tested with the Kruskal-Wallis test (Sokal and Rohlf, 1989) using SAS (Version 8.0 for Windows, SAS Institute, Cary, NC, USA) (SAS, 1999). As there were many 'ties' (due to the prevalence of 100% outcrossing), Monte Carlo simulation of exact tests were used for generation of probability values for the K-W test (SAS, 1999). For all MLTR analyses, all available parameters were simultaneously estimated.

Fruit production

Total number of capsules per sample tree was recorded during each seed collection (open capsules persist for some time, so capsule counts are unbiased by any differences in maturation date). A positive relationship between capsule production and neighbourhood density would suggest pollinator limitation, *i.e.* less visitation. This relationship was tested for using multiple linear regression of the natural log of individual capsule count (averaged over collection years for each tree) on both dbh and individual neighbourhood density index. Dbh was used because of the evident and practically axiomatic relationship between tree size and flower production. The null hypothesis of no between-fragment differences in log capsule production was tested using analysis of covariance (dbh as covariate). Analyses were carried out using SAS.

RESULTS

Genetic variation

Maternal generation

All alleles of all loci were present only in the Quebrada Palmira and Río Corobicí populations (Table 7-3). Fixation of one or more loci occurred in maternal trees sampled in the San Isidro, Praderas (both for PGI), Pachanga (PGM) and Sandillal (AAT, ADH, PGM) populations. For all loci except ADH, the most common allele was the same in each population. For ADH, $p_A > q_B$ in one population, whilst $q>p$ in four populations and $p=q$ in two populations. The frequency of ADH-A varied from $p=0$ (Sandillal) to $p=0.53$ (San Isidro). Gene diversity (H_e)

varied from 0.12 to 0.38. There were two nominally significant Hardy-Weinberg disequilibria (heterozygote excess in Río Corobicí (PGD), and Praderas (ADH)). Twenty-five of the F_{is} values were negative, three were positive, one equal to zero, and seven were undefined (p=1).

The homogeneity tests (Table 7-4) indicate that the populations are heterogenous for ADH and PGI. The overall F_{st} estimate of 0.07 (0.03 without the smallest populations) (Table 7-5) suggests low to moderate genetic differentiation (Yeh 2000). Pairwise genetic distances (Table 6) were significantly positively related to pairwise geographic distances (Mantel test, G=1.60, Z=7.14, r=0.275, one-tailed p=0.036). Populations from Corobicí and its tributaries group together, as do the two River Tenorito populations (Figure 7-2).

Progeny: 1997

All alleles of all loci were present in the Corobicí, Magdalena and San Isidro populations (Table 7-7). PGI-B was absent from Praderas. For all loci except ADH, the same allele was the most common in each population. For ADH, ADH-B was commonest in Corobicí and Magdalena, whilst $p_A \approx q_B$ or $p_A = q_B$ in San Isidro and Praderas. Gene diversity varied from 0.27 (Magdalena) to 0.36 (San Isidro). There were two significant HW disequilibria, both associated with homozygote excess. Most F_{is} estimates were close to zero; five were positive, 15 were negative and one undefined (Table 7-7).

The homogeneity tests (Table 7-8) indicate that sampled populations differ at all loci. The overall F_{st} estimate of 0.07 (0.03 without the smallest populations) (Table 7-9) suggests low to moderate genetic differentiation (Yeh 2000). The relationship between pairwise genetic and geographic distances (Table 7-10) was weak and non-significant (Mantel test: g=0.0531, Z=1.166, r=0.0286). The River Magdalena population grouped separately from the other populations (Figure 7-3).

Progeny: 1998

All alleles were present in all six sampled populations (Table 7-11), except AAT-C (absent in Sandillal) and PGM-C (Quebrada Palmira, Praderas, Sandillal). There was near loss of alleles ($p \leq 0.03$) in Quebrada Palmira (PGM-C), San Isidro (PGM-C), Praderas (PGI-B, PGM-B), Magdalena (AAT-C, PGM-B,C) and Sandillal (AAT-B, ADH-A). For all loci except ADH, the same allele was the most common in each population. For ADH, ADH-B was commonest in Quebrada Palmira, Corobicí, Praderas, Magdalena, Sandillal whilst $p_A=q_B$ in San Isidro. Gene diversity varied from 0.18 (Sandillal) to 0.39 (San Isidro). There were seven significant HW disequilibria, four with homozygote excess and three with heterozygote excess. Eighteen F_{is} estimates were negative and 12 were positive.

Results of the homogeneity tests (not shown) were similar to those for the 1997 data, *i.e.* indicate highly significant heterogeneity for all loci (all probabilities <0.001). The F_{st} estimates of 0.069 for these six populations (Table 7-12) suggest low to moderate genetic differentiation. The relationship between pairwise genetic and geographic distances (Table 7-13) was strongly positive (Mantel test: G=1.858, Z=6.82, r=0.39) and significant (one-tailed p=0.02). The Magdalena and Sandillal populations grouped separately from the remaining populations (Figure 7-4).

Progeny: 1999

All alleles were present in all five sampled populations (Table 7-14), except AAT-C (Sandillal), PGM-C (Magdalena, Pachanga, Sandillal). There was near loss of alleles ($p \leq 0.03$) in Quebrada Palmira (PGM-B, C), Corobicí (PGI-B), Magdalena (AAT-C, PGI-B), Pachanga (PGI-B, PGM-B) and Sandillal (AAT-B, PGM-B). For all loci except ADH, the same allele was the most common in each population. ADH-B was more frequent in Magdalena, Pachanga, and Sandillal, whilst $p_A \approx q_B$ in Quebrada Palmira and Corobicí. Gene diversity varied from 0.14 (Sandillal) to 0.35 (Quebrada Palmira, Corobicí.). There were four significant HW disequilibria, two with homozygote excess and two with heterozygote excess. Seventeen F_{is} estimates were negative, eight were positive, and one was undefined (monomorphism).

The homogeneity tests (not shown) indicated highly significant heterogeneity for all loci (all probabilities <0.001). The F_{st} estimates of 0.09 for these five populations (Table 7-15) suggest moderate genetic differentiation. The relationship between pairwise genetic and geographic distances (Table 7-16) was positive (g=1.18, Z=4.65, r=0.422) and weakly significant (one-tailed p=0.08). The Magdalena and Sandillal populations grouped separately from the remaining populations (Figure 7-5)

Gene flow

For maternal and progeny data, mean estimates of gene flow based on the F_{st} method range from 2-3 individuals generation^{-1} when the Sandillal and Pachanga populations are included, and around 4-7 individuals generation^{-1} when these are omitted (Tables 7-5, 7-9, 7-12, 7-15).

Mean migration rates estimates into the Sandillal population from the Magdalena population were 0.14 and 0.12 in 1998 and 1999, respectively (Table 7-17).

Mating system parameters

In general, estimates of population outcrossing rates were >0.9 (Table 7-18). There was one statistically significant departure (*i.e.* by the $\hat{t} + 2s_{\bar{x}} < 1.0$) criterion (Liengsiri *et al.*, 1998) from 100% outcrossing (Sandillal 1998, $\hat{t} = 0.986 \pm 0.006$). Estimates for Praderas (1998 and pooled data) and Magdalena (1997, 1998, and pooled data) departed more strongly from full outcrossing, but with higher standard errors.

Estimates of population outcrossing rate based on mean single locus values (t_s) were lower than t_m estimates in all cases except three, and in general departed significantly from unity. Estimates of biparental inbreeding based on the difference between these two estimates of the outcrossing rate varied from 1.3% to 6.0%. Estimates of r_t were in the range 0.1-0.25. Most estimates of r_p were close to 0.9, with a minimum of 0.593.

Estimates of individual outcrossing rates ranged from complete selfing to complete outcrossing, but for all populations except Magdalena and Quebrada Palmira, most estimates were >0.9 (Figure 7-6). There were no significant differences between fragments in individual-tree outcrossing rates (Kruskal-Wallis test, chi-square 7.12, $p_{df=6}=0.31$). The scatter plot of individual-tree outcrossing rate against individual neighbourhood density indices (Figure 7-7) did not suggest any clear relationship between the two variables.

Fruit production

The log of number of capsules tree^{-1} was significantly positively related to both dbh and neighbourhood density index (Table 7-19; Figure 7-8) (dbh and density index were not related).

Fragment mean numbers of capsules tree^{-1} varied from 23.0 (Magdalena) to 98.0 (Sandillal) (Table 7-20). After taking into account the effect of diameter variation, there was no significant effect of fragment on mean log numbers of capsules tree^{-1} (Table 7-21), whereas the effect of the covariate was highly significant.

DISCUSSION

Population differentiation

The results indicate that, for these loci, and for the maternal generation, there is low to moderate genetic differentiation between these populations of *P. rubra*. The estimates, although based on relatively few loci, are similar to average G_{st} values for comparable species, *e.g.* long-lived woody species in general (0.084±0.008, Hamrick *et al.*, 1992), and, within this group, outcrossing, animal-pollinated species (0.099±0.017), wind-dispersed species (0.076±0.009), although it is worth noting that Loveless (1992) reports a higher mean value for abiotically dispersed tropical species (0.138±0.026). When the small Sandillal population is omitted from the analysis, the estimate of population differentiation is notably lower (F_{st}=0.035 vs. F_{st}=0.075). The estimates of F_{st} in the 1997 and 1998 progeny cohorts are closely similar to those of the maternal generation. The slightly higher estimates for the 1999 cohort may reflect lower sample sizes.

The results indicate a positive relationship between genetic distance and geographic distance. In the maternal generation, populations from Corobicí and its tributaries group together, as do the two River Tenorito populations. The small, discrete Sandillal population forms a separate group. In the progeny cohorts, the Magdalena population groups separately from both the River Corobicí and Tenorito populations, and is found in the same branch as the geographically relatively close Sandillal population.

Within-population genetic variation

Comparison of levels of within-population gene diversity shown by *P. rubra* with those of published meta-analyses is not straightforward, as the latter (*e.g.* 0.13±0.011 for native tropical woody taxa, Loveless, 1992) are inclusive of monomorphic loci. However, as Loveless reported percentage of loci polymorphic (*P*) of 39.0 per cent for the same group, this implies mean gene diversity of polymorphic loci for this group of (0.13/0.39) = 0.33, i.e. similar to the estimates reported in Tables 7-3, 7-7, 7-11 and 7-14. Similarly, the estimates for *P. rubra* are comparable to other estimates based on polymorphic allozyme loci of neotropical trees, *e.g.* for *Enterolobium cyclocarpum* (H_e=0.365, five loci, Rocha and Lobo, (1996)), *Pithecellobium elegans* (H_e=0.31, recalculated for 6 polymorphic loci, Hall *et al.* (1994c)), *Pentaclethra macroloba* (H_e=0.21, three loci of adult trees, Hall *et al.*, 1994b), *Carapa guianensis* (H_e=0.31, 6 loci, Hall *et al.* 1994a). There is, at least, no reason to conclude from the present data that *P. rubra* shows any general tendency to low levels of genetic diversity.

F_{is} estimates for the maternal generation were generally negative. Heterozygote excess was significant in two cases, in both cases at the two loci found to be non-neutral (Chapter Six). However, the greater prevalence and magnitude of negative F_{is} estimates for the maternal than the progeny generations suggests that heterozygote superiority in later age classes may not be restricted to these two loci. If this is the case, then selection, as well as migration, may contribute to maintaining high genetic variation and low genetic structure in this species.

Although, in general, these *P. rubra* populations have maintained high genetic variation and little divergence, there is, nevertheless, clear evidence of small population effects. The most notable case is that of the Sandillal population, which has retained only about 36% of the gene diversity (H_e) of surrounding populations. In part, this may reflect founder effects at fragmentation. This hypothesis is supported to some degree by the allelic richness of the Sandillal population (A=1.4). The expected average number of alleles remaining after a founder event is:

$$E = A - \sum_j \left(1 - p_j\right)^{2N},$$

where A = the number of alleles before population reduction, p_j =frequency of the jth allele, N=population size after reduction (Meffe and Carroll, 1994). Assuming pre-bottleneck frequencies equivalent to average frequencies for the Magdalena population (*i.e.*, p=0.85, q=0.13, r=0.02 (AAT); 0.23, 0.77 (ADH); 0.95, 0.05 (PGI), 0.965, 0.03, 0.005 (PGM); 0.73, 0.27 (PGD)), by this formula expected mean allelic richness for a 'sample' of four trees is $\hat{A} = 1.64$, *i.e.* not very different from the observed value of 1.40.

However, in general founder effects on gene diversity are less intense (Frankel and Soulé, 1981). Expected change in gene diversity as a result of a population size reduction (Young *et al.* (1996)) is:

$$\Delta H_e = \frac{-H_e}{S},$$

where S = number of gametes. In the present case ($H_e \approx 0.32$, S=8), the expected loss of 0.04 is much less then the observed loss of around 0.2, implying that additional factors have contributed to the loss of variation. One possibility is subsequent genetic drift. As expected gene diversity after *t* generations is approximated by:

$$H_e = H_0 e^{-\left(\frac{t}{2N}\right)},$$

where N = effective population size and H_0= initial gene diversity, the number of generations required for a given change ΔH_e can be estimated using:

$$-t = (2N)\ln\frac{H_e}{H_0} \text{ (Hartl and Clark, 1989).}$$

Assuming constant effective population size of $N=N_e=4$, the proportion of gene diversity lost in excess of that predicted due to founder effects (*i.e.* 0.12/0.28) would require (*ln*0.43)(8) or 7 generations. Although, given the short generation time of *P. rubra*, this number of generations does not seem too high to explain the observations, there may also be other factors at work, *e.g.* $N_e<N$ due to greater than expected variation in fecundity, overlapping generations, biparental inbreeding and possibly clumped population structure due to restricted seed dispersal.

Loss of genetic variation is of particular interest in the case of the locus ADH, which appears to be subject to balancing selection (*i.e.* heterozygote advantage) in the adult or older juvenile phases (see Chapter Six). Such loci are of intrinsic interest, *i.e.* not solely as 'markers'. Fixation has occurred at ADH*3* (and, therefore, the closely linked ADH*2* (Chapter Six)). Depending on the nature and presence in this population of the environmental factor(s) responsible for the selection pressure, this may imply a loss in population fitness, which will be permanent unless restored by migration or, improbably, by mutation.

There is some additional evidence for genetic drift effects on genetic variation. In all progeny years, the Magdalena and Praderas populations show lower gene diversity values than the other populations (except Sandillal). Praderas is the most isolated of all the populations (Figure 7-1), whilst the Magdalena population is located in a semi-urban and highly-disturbed setting, and both have relatively low population sizes. Relatively low gene diversity for the non-neutral ADH was also observed in the case of the Magdalena population (H_e=0.39, compared to $H_e \approx 0.5$ for the larger populations).

Gene flow

Its significance notwithstanding, the low-to-moderate degree of subpopulation differentiation described here suggest that to date, gene flow between these populations of *P. rubra* has been substantial. Overall estimates of gene flow based on the Island Model suggest an average of 2-7 migrants generation^{-1}. Given the failures in assumptions of the Island Model in the present case, it is possible that this range underestimates true values.

The direct estimates of current gene flow provide partial support for this argument. If applicable to other populations, the observed migration rate of 0.13 into the Sandillal population would imply mN large enough to restrict population divergence to (e.g. for N=50, mN=6.5, corresponding to equilibrium F_{st} of $\frac{1}{1+4mN}$ =0.037). However, in the case of the smaller populations, such as Magdalena (N=20), such levels of gene flow, when combined with factors likely to reduce N_e relative to N, may nevertheless be inadequate to prevent population divergence. The difference in grouping of the Magdalena population in progeny and adult generations, as well as the skewed frequencies of ADH in this population, seems to support this conjecture. In the case of Sandillal, quite extensive gene flow has demonstrably been insufficient to prevent divergence. As 26 per cent pollen flow corresponds to 0.5 migrants per generation, this is in accordance with theoretical predictions of substantial divergence at mN<1, even taking into account small population size (*i.e.* using Wright's (1969) full equation). The case of the Sandillal population is perhaps an extreme one, both in terms of isolation and small population size. However, slightly larger populations such as Magdalena are not uncommon. Given the expectation that $N_e < N$, such populations could be at risk from genetic drift effects.

Mating system parameters

The results support Haber's (1984) deduction that, like many tropical trees, *P. rubra* is self-incompatible. Only one population (Sandillal) showed significant departure from full outcrossing in any year, and the estimate in question is very close to 100 per cent, *i.e.* of questionable biological importance. However, significant biparental inbreeding, *i.e.* mating between relatives, was noted in at least one year in all populations except Praderas. A number of factors could promote biparental inbreeding in *P. rubra*, *e.g.* limited seed dispersal ability, clumped habitat, and high correlation of paternity, itself due to uniparental or close to uniparental paternity of individual capsules. However, there is little indication of accumulation of inbreeding in the F_{is} estimates, which tend to be strongly negative in adult populations. This may indicate that inbreeding depression acts to purge progeny of related individuals, *i.e.* of full sibs, as has been speculated for the colonizer *Centaurea solstitialis* (Sun and Ritland, 1998). Differences between fragments in biparental inbreeding tend to be small and non-significant (*i.e.* within the limits of the respective standard errors), and therefore preclude the direct establishment of relationships between degree of biparental inbreeding and fragment characteristics.

The estimates of correlation of paternity suggest strongly that individual capsules of *P. rubra* tend to be sired by one or few pollen parents. A similar finding was reported for the apocynaceous *Stemmadennia donnell-smithii* (James *et al.*, 1998), and is consistent with the observed low frequency of pollinator visits (Haber, 1984).

There were a number of cases of individual outcrossing estimates <100 per cent, including two apparent cases of complete selfing. Such apparent departures from full outcrossing in individuals of a self-incompatible (or any other) species occur when fertilizing gametes have haploid multilocus genotypes that could be produced by the tree under study. This could occur either as a result of self-fertilization or as a result of fertilization by another tree with the same multilocus genotype or, at least, with ≥1 allele in common at each locus. Normally, such events would be unusual, *i.e.* confined to individual zygotes within progeny arrays. However, if all seeds within capsules are full-sibs, then each capsular array is analogous to each element (zygote) of a non-correlated array, in the sense that each represents an observation of a single pollination event. However, they are distinct in the sense that there is a higher probability of unambiguous designation of the pollination event as an outcross, as the detection of even one unambiguous outcross in a full-sib capsular array would permit designation of the entire array as outcrossed. Many, and perhaps all, of the cases of apparent departure from complete outcrossing reported here may be due to biparental inbreeding. For example, Tree QP2, one of the two individuals with $\hat{t} = 0$, has inferred multilocus genotype AAT-AA, ADH-BB, PGI-AA, PGM-AA, PGD-AB. Its 24 progeny, all derived from one capsule, are all homozygous for the first four loci. Clearly, in the Quebrada Palmira population, which has relatively high diversity at the first three loci (Table 7-3), this array must either be due to selfing or pollination by one, similar genotype. Tree QP 1-309 has the same multilocus genotype, whilst the only progeny collected from the nearby tree QP4 has genotype consistent with the same maternal genotype. Based on these considerations, and given the apparent presence of an incompatibility mechanism in *P. rubra,* it seems reasonable to suggest that biparental inbreeding, rather than breakdown of self-incompatibility, represents the most parsimonious explanation of the observed departures from 100 per cent outcrossing.

The individual outcrossing rates provide additional confirmation of abundant potential gene flow in *P. rubra.* The progeny arrays assayed derived from the most isolated mother-trees (Palmira cemetery tree (nearest neighbour 390m), River Corobicí Tree 1000 (nearest neighbour at 318m), and River Corobicí Tree 1011 (nearest neighbour 190m) had $\hat{t} = 1.0$, $\hat{t} = 1.0$ and $\hat{t} = 0.9$, respectively, in spite of their high degree of isolation.

Fruit production

The estimated regression coefficient for NDI (0.056) indicates that increases in 1 unit of NDI (equivalent, for example, to 1 additional tree within 25m, or 4 additional trees between 50m and 100m) is associated with a multiplicative change in the median number of capsules for the NDI in question of $e^{0.056}$, *i.e.* 5.8% (with lower and upper 95% confidence limits of $e^{0.0002} \approx 0\%$ and $e^{0.101} = 10.6\%$ (Ramsey and Schafer, 1997). For dbh=30 cm and NDI=0.25, predicted number of capsules is 23.66, whilst at NDI 4.0, predicted number of capsules is 29.2. The estimated slope of the relationship between NDI and capsules is the same as for dbh, although with wider confidence limits.

As outlined above, high outcrossing rates even in highly isolated trees indicate that the hawkmoth pollinators of *P. rubra* reach even highly isolated trees. However, the relationship with NDI seems to suggest that the probability of them so doing is related to the degree of individual isolation, as is suggested also by the low genetic variation of the Sandillal population. This has implications for the effects of fragmentation on genetics of the species. Isolated populations, insofar as these are located in fragments with low resident population of pollinating species, will suffer reduced population fitness due to lower fertility. The same will occur in the case of low density populations, *e.g.* as the result of fragmentation-mediated disturbance.

Implications

Hawkmoths are known to be strong fliers and effective pollinators. *P. rubra*, a 'naturally-fragmented', hawkmoth-pollinated tree species, shows little sign of exhibiting unusually low within-population variation or high inter-population differentiation at the scale examined here, *i.e.* that appropriate to the spatial scale of the forest fragmentation itself. The lack of strong population differentiation exhibited by the species is indicative of substantial between fragment gene flow. Given the relatively low probability of long-distance seed dispersal, it seems reasonable to conclude that the nature of its pollinator is in large measure responsible for the apparent robustness to small population effects of *Plumeria rubra.* The self-incompatibility of the species, especially given the presence of pollinator limitation, also seems to contribute to this robustness.

Fundamentally, species occupy fragmented habitat because they are able to compete, grow and persist in such habitat. It appears that, in the case of *P. rubra,* this ability is conferred partially by the pollination system. Such species characteristics could be considered as constituting a variety of 'preadaptation' to habitat fragmentation. However, the resilience of this system is not inexhaustible, and, in certain cases, thresholds may be crossed beyond which adverse consequences may ensue. Some such consequences are illustrated by the present study, *e.g.* loss of genetic variation and declines in population fitness, as evidenced both by fixation at non-neutral loci and by reduction in fertility of isolated trees.

P. rubra appears to have a rather finely balanced pollination system: some of its characteristics enable it to overcome apparent isolation, whilst others appear to potentially increase its vulnerability to fragmentation. The potent scent-production of its flowers enables it to attract pollinators at distance, and its spectacular floral displays aids visual location even at night. Its nectarless flowers presumably promote inter-tree pollinator movement (a goal possibly secured in nectar-producing mass-flowering species by inter-tree variation in nectar production (Frankie and Haber, 1983)) and may discourage repetitive crossing patterns, as might occur in 'traplining' behaviour in linear habitat (Janzen, 1974). However, as Haber (1984) pointed out, the effectiveness of its deceitful, imitative pollination system may be partially dependent on the persistence in increasingly degraded habitat of other, nectar-producing species. Consequently, although, to date, the continued persistence of this species in its favoured habitat seems not be at risk due to deforestation, fragmentation and disturbance, there is no guarantee that this will continue to be the case.

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Table 7-1. Description of populations sampled in a study of the genetic effects of forest fragmentation on *Plumeria rubra* in northwestern Costa Rica.

Population	Latitude, Longitude[1]	N[2]	isolation[3]	disturbance[4]	matrix	ndi[5] ($\bar{x}$,CV%)	dbh[6] ($\bar{x}$,CV%)	Comments
Quebrada Palmira	85°05.7' 10°32.3'	<30	L	L-M	pastureland	0.95,127	29.6,39	Includes two outlying trees, probably planted, in Palmira cemetery and neighbouring pasture
River Corobicí	85°05.9' 10°32.7'	22	L	L-M	pastureland	2.15,82	32.2,48	
San Isidro	85°08.5' 10°30.0'	35 (300+)	H	L (M-H)	pastureland, open forest	7.76,44	29.2,35	upstream of sampled area, there is open forest with individual *P. rubra* trees until a large concentration of *P. rubra* located near Panales (Figure 5-1)
Praderas del Norte	85°08.6' 10°31.9'	30-50	H	L (M)	plateau pastureland, open forest	2.2[7]	22[8]	
Magdalena	85°06.6' 10°28.5'	21	H	H (M)	mixed agriculture, pastureland, rural dwellings	2.31,72%	21.9,54	

[1]At population centre; [2]Population size; [3]L=low, M=moderate, H=high (qualitative ranking based on distance to and size of nearest neighbouring fragment); [4]L=low, M=moderate, H=high, based on tree cover of fragment where population is located, parentheses refer to localized atypical sectors; [5]neighbourhood density index, see text; [6]diameter at breast height; [7]estimate; [8]estimate based on mean fruit production tree^{-1}

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Table 7-2. Sampling schedule in a study of genetic effects of forest fragmentation in *Plumeria rubra* in northwestern Costa Rica

	Collection			
Fragment, sampling descriptors	**1997**	**1998**	**1999**	**Pooled**
Quebrada Palmira				
Number of families assayed	1	6	11	15
Number of progeny assayed	21	147	188	356
Mean number progeny family^{-1}	21	24.5	17.1	24.1
Mean number of capsules family^{-1}	5	3.3	3.7	4.4
Mean number progeny capsule^{-1}	5.2	8.2	5.6	6.7
Río Corobicí				
Number of families assayed	15	16	9	17
Number of progeny assayed	381	320	170	871
Mean number progeny family^{-1}	25.4	20	18.9	51.1
Mean number of capsules family^{-1}	3.7	3.6	3.7	8.5
Mean number progeny capsule^{-1}	9.4	7.3	5.3	7.4
San Isidro				
Number of families assayed	11	16	0	17
Number of progeny assayed	205	255	0	460
Mean number progeny family^{-1}	18.6	15.9	0	27.1
Mean number of capsules family^{-1}	3.3	3.1	0	5.1
Mean number progeny capsule^{-1}	5.8	4.9	0	4.7
Praderas				
Number of families assayed	8	16	0	16
Number of progeny assayed	170	310	0	480
Mean number progeny family^{-1}	24.3	20.5	0	27.0
Mean number of capsules family^{-1}	3.1	3.4	0	4.5
Mean number progeny capsule^{-1}	7.1	5.0	0	5.4
Magdalena				
Number of families assayed	15	12	5	17
Number of progeny assayed	364	246	111	721
Mean number progeny family^{-1}	24.3	20.5	22.2	42.0
Mean number of capsules family^{-1}	3.4	3.3	3.8	6.5
Mean number progeny capsule^{-1}	7.4	6.4	6.3	7.2
Pachanga				
Number of families assayed	0	0	8	8
Number of progeny assayed	0	0	171	171
Mean number progeny family^{-1}	0	0	21.4	21.4
Mean number of capsules family^{-1}	0	0	4.1	4.1
Mean number progeny capsule^{-1}	0	0	5.3	5.3

Table 7-2. Sampling schedule in a study of genetic effects of forest fragmentation in *Plumeria rubra* in northwestern Costa Rica (continued)

Fragment, sampling descriptors	Collection			
	1997	**1998**	**1999**	**Pooled**
Sandillal				
Number of families assayed	0	3	4	4
Number of progeny assayed	0	127	86	213
Mean number progeny family^{-1}	0	42.3	21.5	53.2
Mean number of capsules family^{-1}	0	4.7	5.2	8.8
Mean number progeny capsule^{-1}	0	9.2	4.2	6.0
All				
Number of families assayed	50	69	37	94
Number of progeny assayed	1141	1405	726	3272
Mean number progeny family^{-1}	22.8	20.4	19.6	34.3
Mean number of capsules family^{-1}	3.4	3.4	4.0	5.8
Mean number progeny capsule^{-1}	7.6	6.2	5.4	6.2

Table 7-3. Estimates of allele frequencies, number of alleles, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in populations of *P. rubra* from 7 forest fragments in northwestern Costa Rica, based on inferred maternal genotypes.

Population / Locus	Allele frequencies			A[1]	H_e[2]	F_{is}[3]
	p[4]	q[4]	r[4]			
Quebrada Palmira						
AAT	0.69	0.23	0.07	3	0.48	-0.08
ADH	0.54	0.46	n.a.	2	0.50	-0.01
PGI	0.82	0.18	n.a	2	0.29	-0.22
PGM	0.95	0.035	0.035	3	0.14	-0.08
PGD	0.50	0.50	n.a	2	0.50	-0.38
means				2.40(.55)	0.38(0.16)	
River Corobicí						
AAT	0.79	0.15	0.06	3	0.34	0.10
ADH	0.35	0.65	n.a.	2	0.46	-0.29
PGI	0.97	0.03	n.a.	2	0.06	-0.03
PGM	0.88	0.03	0.08	3	0.22	-0.13
PGD	0.56	0.44	n.a.	2	0.49	-0.55*
means				2.4 (.55)	0.31 (0.18)	
San Isidro						
AAT	0.71	0.18	0.12	3	0.46	-0.13
ADH	0.50	0.50	n.a.	2	0.50	-0.11
PGI	1.00	0.0	n.a.	1	0.00	M
PGM	0.78	0.19	0.03	3	0.36	0.04
PGD	0.61	0.39	n.a.	2	0.48	-0.17
means				2.20 (.84)	0.36 (0.21)	
Praderas						
AAT	0.69	0.06	0.25	3	0.46	-.08
ADH	0.44	0.56	n.a.	2	0.49	-.42*
PGI	1.00	0.0	n.a.	1	0.00	M
PGM	0.88	0.12	0.0	2	0.22	-.13
PGD	0.75	0.25	n.a.	2	0.38	0.00
means				2.0 (0.71)	0.31(0.20)	
Magdalena						
AAT	0.79	0.18	0.03	3	0.34	-0.26
ADH	0.26	0.74	n.a.	2	0.39	-0.36
PGI	0.91	0.08	n.a.	2	0.16	-0.10
PGM	0.88	0.12	0.0	2	0.21	-0.13
PGD	0.65	0.35	n.a.	2	0.46	-0.29
Mean				2.20 (0.45)	0.31(.12)	

[1]Allelic richness; [2]Nei's expected heterozygosity; [3]Asterisks indicate significance of G-test for Hardy-Weinberg disequilibrium: ***p<.001, **<.01, *>.05; M=monomorphic; [4]frequencies of alleles A, B, C, respectively

Table 7-3. Estimates of allele frequencies, number of alleles, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in populations of *P. rubra* from 7 forest fragments in northwestern Costa Rica, based on inferred maternal genotypes (continued).

Population / Locus	Allele frequencies			A^1	$H_e{}^2$	$F_{is}{}^3$
	p^4	q^4	r^4			
Pachanga						
AAT	0.75	0.19	0.06	3	0.40	0.33
ADH	0.50	0.50	n.a.	2	0.50	-0.50
PGI	0.94	0.06	n.a.	2	0.12	-0.07
PGM	1.0	0.0	0.0	1	0.00	M
PGD	0.50	0.50	n.a.	2	0.50	-0.50
Mean				2.0 (0.71)	0.30(0.23)	
Sandillal						
AAT	1.0	0.0	0.0	1	0.00	M
ADH	0.0	1.0	n.a.	1	0.00	M
PGI	0.88	0.12	n.a.	2	0.22	-.14
PGM	1.0	0.0	0.0	1	0.00	M
PGD	0.75	0.25	N.A	2	0.38	-0.33
Mean				1.4 (0.55)	0.12(0.17)	

[1]Allelic richness; [2]Nei's expected heterozygosity; [3]Asterisks indicate significance of G-test for Hardy-Weinberg disequilibrium: ***p<.001, **<.01, *>.05; M=monomorphic; [4]frequencies of alleles A, B, C, respectively

Table 7-4. Results of chi-square and likelihood ratio (G) tests of homogeneity of allele frequencies at five loci of seven populations of *Plumeria rubra* in northwestern Costa Rica (based on inferred maternal genotypes)

Locus	chi-square, df, probability	G, df, probability
AAT	4.9, 6, 0.56	6.8, 6, 0.34
ADH	12.7, 6, 0.05	15.6, 6, 0.02
PGI	13.2, 6, 0.04	14.7, 6, 0.02
PGM	7.6, 6, 0.27	9.8, 6, 0.13
PGD	6.0, 6, 0.42	6.14, 6, 0.441

Table 7-5. Estimates of Wright's statistics and mN between seven populations (inferred maternal genotypes) (estimates in parentheses based on data set without Pachanga and Sandillal populations)

Locus	Sample size	F_{is}[1]	F_{it}[2]	F_{st}[3]	mN[4]
AAT	184 (160)	-0.0951 (-0.1248)	-0.0322 (-0.0946)	0.0575 (0.0269)	4.1 (9.0)
ADH	188 (164)	-0.2954 (-0.2516)	-0.1254 (-0.2015)	0.1312 (0.0400)	1.6 (6.0)
PGI	188 (164)	-0.1418 (-0.1586)	-0.0742 (-0.0630)	0.0592 (0.0825)	4.0 (2.8)
PGM	188 (164)	-0.1074 (-0.1074)	-0.0371 (-0.0728)	0.0635 (0.0312)	3.7 (7.8)
PGD	186 (162)	-0.3309 (-0.2938)	-0.2770 (-0.2549)	0.0404 (0.0312)	5.9 (8.1)
Mean	187 (163)	-0.2225 (-0.2066)	-0.1313 (-0.1638)	0.0747 (0.0354)	3.1 (6.8)

[1]correlation between uniting gametes relative to the subpopulations; [2]correlation between uniting gametes relative to the population as a whole; [3]correlation between two gametes drawn randomly within subpopulations relative to that of two gametes drawn randomly from the population as a whole; [4]estimated number of migrants generation^{-1} ($=0.25(1-F_{st})/F_{st}$)

Table 7-6. Pairwise estimates of Nei's unbiased genetic distance (below diagonal) and geographic distances (km, above diagonal) between seven populations of *Plumeria rubra* located in Guanacaste province, northwestern Costa Rica

	Q. Palmira	**R. Corobicí**	**San Isidro**	**Praderas**	**Magdalena**	**Pachanga**	**Sandillal**
Q. Palmira		0.99	6.574	4.119	7.464	5.914	8.733
R. Corobicí	0.011		6.472	3.423	8.072	5.727	8.66
San Isidro	0.013	0.008		3.593	6.472	3.959	6.454
Praderas	0.033	0.017	0.006		8.429	5.646	8.745
Magdalena	0.026	0.001	0.017	0.018		1.85	1.304
Pachanga	-0.01	0.001	0.007	0.023	0.017		3.129
Sandillal	0.097	0.041	0.088	0.065	0.019	0.079	

Table 7-7. Estimates of allele frequencies, number of alleles, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in 1997 progeny of four populations of *P. rubra* in northwestern Costa Rica

Population / Locus	**Allele frequencies**			**A**[1]	**H_e**[2]	**F_{is}**[3]
	p[4]	q[4]	r[4]			
Río Corobicí						
AAT	0.77	0.17	0.06	3	0.37	0.04
ADH	0.40	0.60	n.a.	2	0.48	0.05
PGI	0.96	0.04	n.a.	2	0.08	0.15*
PGM1	0.88	0.04	0.07	3	0.21	-0.04
PGD	0.61	0.39		2	0.48	-0.05
Means (s.d.)				2.4 (0.55)	0.37 (0.20)	
San Isidro						
AAT	0.76	0.18	0.06	3	0.39	-0.06
ADH	0.51	0.49	n.a.	2	0.50	-0.04
PGI	0.96	0.04	n.a.	2	0.08	-0.05
PGM1	0.79	0.17	0.04	3	0.35	-0.03
PGD	0.54	0.46	n.a.	2	0.50	0.03
Means (s.d.)				2.4 (0.55)	0.36 (0.15)	
Praderas						
AAT	0.77	0.06	0.17	3	0.38	-0.07
ADH	0.48	0.51	n.a.	2	0.50	-0.02
PGI	1.00	0.0	n.a	1	0.00	M
PGM	0.90	0.09	0.01	3	0.18	-0.09
PGD	0.78	0.22	n.a.	2	0.34	-0.03
Means (s.d.)				2.20 (0.84)	0.31 (0.19)	
Magdalena						
AAT	0.78	0.19	0.03	3	0.36	0.10*
ADH	0.21	0.79	n.a.	2	0.34	-0.03
PGI	0.93	0.07	n.a.	2	0.12	-0.03
PGM	0.96	0.04	0.005	3	0.08	-0.04
PGD	0.69	0.31	n.a.	2	0.42	0.01
Means (s.d.)				2.4 (0.55)	0.32 (.19)	

[1] Allelic richness; [2] Nei's expected heterozygosity; [3] Asterisks indicate significance of G-test for Hardy-Weinberg disequilibrium: ***p<.001, **<.01, *>.05; M=monomorphic; [4] frequencies of alleles A, B, C, respectively

Table 7-8. Results of chi-square and likelihood ratio (G) tests of homogeneity of allele frequencies at five loci of four populations of *Plumeria rubra* in northwestern Costa Rica (1997 progeny)

Locus	chi-square, df, probability	G, df, probability
AAT	22.20, 3, <0.000	24.8, 3, <0.000
ADH	128.5, 3, <0.000	132.5, 3, <0.000
PGI	21.2, 3, <0.000	32.8, 3, <0.000
PGM1	88.0, 3, <0.000	73.9, 3, <0.000
PGD	41.2, 3, <0.000	42.1, 3, <0.000

Table 7-9. Estimates of Wright's statistics and *mN* between 1997 progeny of four populations of *P. rubra* located in Guanacaste province, northwestern Costa Rica.

Locus	Sample size	F_{is}[1]	F_{it}[2]	F_{st}[3]	*mN*[4]
AAT	2098	-0.0381	-0.0234	0.0142	17.35
ADH	2132	-0.0070	0.0484	0.0550	4.29
PGI	2244	0.0158	0.0313	0.0158	15.60
PGM	2252	-0.0534	-0.0169	0.0347	6.96
PGD	1778	-.0070	0.0303	0.0371	6.49
Mean	2101	-0.0189	0.0172	0.0355	6.70

[1]correlation between uniting gametes relative to the subpopulations; [2]correlation between uniting gametes relative to the population as a whole; [3]correlation between two gametes drawn randomly within subpopulations relative to that of two gametes drawn randomly from the population as a whole; [4]estimated number of migrants generation^{-1} (=0.25(1-F_{st})/F_{st})

Table 7-10. Pairwise estimates of Nei's unbiased genetic distance (below diagonal) and geographic distances (km, above diagonal) between 1997 progeny of four populations of *Plumeria rubra* located in Guanacaste province, northwestern Costa Rica

	R. Corobicí	San Isidro	Praderas	Magdalena
R. Corobicí		6.472	3.423	8.072
San Isidro	0.0087		3.593	6.472
Praderas	0.0144	0.0232		8.429
Magdalena	0.0120	0.0375	0.0280	

Table 7-11. Estimates of allele frequencies, number of alleles, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in 1998 progeny of six populations of *P. rubra* in northwestern Costa Rica

Population / Locus	Allele frequencies			A[1]	H_e[2]	F_{is}[3]
	p[4]	q[4]	r[4]			
Qbrda. Palmira						
AAT	0.65	0.29	0.06	3	0.49	-0.08
ADH	0.41	0.59	n.a.	2	0.48	0.02
PGI	0.86	0.14	n.a.	2	0.24	0.36***
PGM	0.97	0.03	0.0	2	0.06	-0.03
PGD	0.55	0.45	n.a.	2	0.50	-0.20*
means				2.2(0.45)	0.36 (0.20)	
Río Corobicí						
AAT	0.81	0.15	0.04	3	0.31	0.13*
ADH	0.38	0.62	n.a.	2	0.47	0.09
PGI	0.94	0.06	n.a.	2	0.12	-0.07
PGM	0.89	0.04	0.07	3	0.20	-0.09
PGD	0.52	0.48		2	0.50	0.01
means				2.4 (0.55)	0.32 (0.16)	
San Isidro						
AAT	0.62	0.25	0.13	3	0.53	-0.12
ADH	0.50	0.50	n.a.	2	0.50	0.05
PGI	0.93	0.07	n.a.	2	0.13	-0.07
PGM	0.84	0.13	0.02	3	0.27	-0.16**
PGD	0.53	0.47		2	0.50	-0.11
means				2.4 (0.55)	0.39 (.18)	
Praderas						
AAT	0.65	0.05	0.30	3	0.49	0.13*
ADH	0.43	0.57	n.a.	2	0.49	0.01
PGI	0.99	0.01	n.a	2	0.02	-0.01
PGM	0.97	0.03	0.0	2	0.06	-0.03
PGD	0.74	0.26	n.a.	2	0.38	-0.08
means				2.20 (0.45)	0.29 (0.23)	
Magdalena						
AAT	0.83	0.16	0.01	3	0.29	0.21**
ADH	0.23	0.76	n.a.	2	0.36	0.04
PGI	0.94	0.06	n.a.	2	0.12	0.02
PGM	0.986	.005	0.009	3	0.03	-0.01
PGD	0.66	0.34		2	0.45	0.01
Mean				2.44 (0.55)	0.25(.17)	
Sandillal						
AAT	0.98	0.02	0.0	2	0.04	-0.02
ADH	0.03	0.97	n.a.	2	0.06	-0.03
PGI	0.85	0.15	n.a.	2	0.25	-0.17*
PGM	0.96	0.04	0.0	2	0.08	-0.04
PGD	0.64	0.36	N.A.	2	0.46	-0.16
Mean				2.0 (0.00)	0.18(.18)	

[1]Allelic richness; [2]Nei's expected heterozygosity; [3]Asterisks indicate significance of G-test for Hardy-Weinberg disequilibrium: ***p<.001, **<.01, *>.05; M=monomorphic; [4]frequencies of alleles A, B, C, respectively

Table 7-12. Estimates of Wright's statistics and mN between 1998 progeny of six populations of *P. rubra* located in Guanacaste province, northwestern Costa Rica.

Locus	Sample size	F_{is}[1]	F_{it}[2]	F_{st}[3]	mN[4]
AAT	2356	0.0218	0.1118	0.0919	2.46
ADH	2902	0.0391	0.1437	0.1089	2.04
PGI	2704	0.0304	0.0601	0.0306	7.91
PGM	2732	-0.0837	-0.0405	0.0399	6.02
PGD	2346	-0.0900	-0.0595	0.0279	8.69
Means	2608	-0.0161	0.0539	0.0689	3.4
Means without Sandillal	2396	-0.0036	0.0358	0.0393	6.11

[1]correlation between uniting gametes relative to the subpopulations; [2]correlation between uniting gametes relative to the population as a whole; [3]correlation between two gametes drawn randomly within subpopulations relative to that of two gametes drawn randomly from the population as a whole; [4]estimated number of migrants generation^{-1} (=$0.25(1-F_{st})/F_{st}$)

Table 7-13. Pairwise estimates of Nei's unbiased genetic distance (below diagonal) and geographic distances (km) between six populations of *P. rubra* located in Guanacaste province, Costa Rica

	Q. Palmira	R. Corobicí	San Isidro	Praderas	Magdalena	Sandillal
Q. Palmira		0.99	6.574	4.119	7.464	8.733
R. Corobicí	0.0110		6.472	3.423	8.072	8.66
San Isidro	0.0089	0.0138		3.593	6.472	6.454
Praderas	0.0325	0.0336	0.0274		8.429	8.745
Magdalena	0.0195	0.0123	0.0351	0.0307		1.304
Sandillal	0.0626	0.0415	0.0897	0.0763	0.0174	

Table 7-14. Estimates of allele frequencies, number of alleles, expected heterozygosity, fixation indices and G-test for Hardy-Weinberg disequilibria in 1999 progeny of five populations of *P. rubra* in northwestern Costa Rica

Population / Locus	**Allele frequencies**			**A**^{1}	**H**$_e$^{2}	**F**$_{is}$^{3}
	p^4	q^4	r^4			
Qbrda. Palmira						
AAT	0.68	0.22	0.09	3	0.47	0.10
ADH	0.52	0.48	n.a.	2	0.50	0.17*
PGI	0.90	0.10	n.a.	2	0.19	0.00
PGM	0.95	0.03	0.02	3	0.10	0.06
PGD	0.61	0.39		2	0.48	0.02
means				2.4 (0.55)	0.35 (0.19)	
Río Corobicí						
AAT	0.76	0.16	0.08	3	0.39	-0.20
ADH	0.48	0.52	n.a.	2	0.50	-0.42***
PGI	0.99	0.01	n.a.	2	0.01	0.00
PGM	0.86	0.07	0.07	3	0.24	-0.05
PGD	0.59	0.41		2	0.48	-0.09
means				2.4 (.55)	0.32 (0.21)	
Magdalena						
AAT	0.95	0.04	0.01	3	0.09	-0.05
ADH	0.25	0.75	n.a.	2	0.37	-0.03
PGI	0.97	0.03	n.a.	2	0.06	-0.03
PGM	0.95	0.05	0.0	2	0.10	-0.05
PGD	0.84	0.16		2	0.27	-0.10
Mean				2.2 (0.45)	0.18(.14)	
Pachanga						
AAT	0.75	0.17	0.08	3	0.40	-0.09
ADH	0.42	0.58	n.a.	2	0.49	-0.20**
PGI	0.99	0.01	n.a.	2	0.02	0.66**
PGM	0.98	0.02	0.0	2	0.04	-0.02
PGD	0.44	0.56		2	0.49	0.15
Mean				2.20 (0.45)	0.29(.24)	
Sandillal						
AAT	0.97	0.03	0.0	2	0.06	-0.03
ADH	0.04	0.96	n.a.	2	0.08	-0.04
PGI	0.91	0.09	n.a.	2	0.16	-0.09
PGM	0.98	0.02	0.0	2	0.04	-0.02
PGD	0.75	0.25		2	0.37	-0.18
Mean				2.0 (0.0)	0.14(.14)	

[1] Allelic richness; [2] Nei's expected heterozygosity; [3] Asterisks indicate significance of G-test for Hardy-Weinberg disequilibrium: ***p<.001, **<.01, *>.05; M=monomorphic; [4] frequencies of alleles A, B, C, respectively

Table 7-15. Estimates of Wright's statistics and mN between 1999 progeny of five populations.

Locus	Sample size	F_{is}^{1}	F_{it}^{2}	F_{st}^{3}	mN^{4}
AAT	1294	-0.0417	0.0299	0.0687	3.39
ADH	1332	-0.1197	0.0347	0.1379	1.56
PGI	1402	-0.0126	0.0252	0.0373	6.45
PGM	1360	-0.0028	0.0244	0.0271	8.98
PGD	1072	-0.0283	0.0570	0.0829	2.76
Mean	1292	-0.0559	0.0393	0.0902	2.52
Mean without Sandillal, Pachanga	793	-0.0513	0.0053	0.0538	4.39

[1]correlation between uniting gametes relative to the subpopulations; [2]correlation between uniting gametes relative to the population as a whole; [3]correlation between two gametes drawn randomly within subpopulations relative to that of two gametes drawn randomly from the population as a whole; [4]estimated number of migrants generation^{-1} (=0.25(1-F_{st})/F_{st})

Table 7-16. Pairwise estimates of Nei's unbiased genetic distance (below diagonal) and geographic distances (km) between five populations of P. rubra located in Guanacaste province, Costa Rica.

	Q. Palmira	R. Corobicí	Magdalena	Pachanga	Sandillal
Q. Palmira		0.99	7.464	5.914	8.733
R. Corobicí	0.0058		8.072	5.727	8.66
Magdalena	0.0452	0.0355		1.85	1.304
Pachanga	0.0137	0.0088	0.0551		3.129
Sandillal	0.0769	0.0643	0.0122	0.0688	

Table 7-17. Estimates of current (1998, 1999) gene flow from the Magdalena to Sandillal populations based on four alleles absent from the Sandillal population

Allele	q_s^{1}	q_t^{2} 1998	m^{3} (1998)	q_t 1999	m (1999)
AAT-B	0.18	0.02	0.11	0.03	0.17
AAT-C	0.03	0	0	0	0
ADH-A	0.26	0.03	0.12	0.04	0.15
PGM-B	0.12	0.04	0.33	0.02	0.17
Mean			0.14		0.12
Standard deviation			0.14		0.08

[1]allele frequency in Magdalena population; [2]allele frequency in Sandillal progeny; [3]m=proportion of immigrant alleles, i.e. q_t/q_s (see text)

Table 7-18. Estimates of mating system parameters in seven population of *Plumeria rubra* located in forest fragments in northwestern Costa Rica

Population / year	F $(s_{\bar{x}})^1$	$t_m (s_{\bar{x}}.)^2$	$t_s (s_{\bar{x}})^3$	tm-ts $(s_{\bar{x}})^4$	$r_t (s_{\bar{x}})^5$	$r_p (s_{\bar{x}})^6$
Quebrada Palmira						
pooled data	0.001 (0.001)	0.944 (0.076)	0.894 (0.068)	0.050 (0.025)	0.192 (0.114)	0.99 (0.038)
1998	0.002 (0.001)	0.997 (0.026)	0.945* (0.019)	0.052 (0.019)	0.105 (0.014)	0.99 (0.043)
1999	0.001 (0.102)	0.944 (0.083)	0.894 (0.065	0.050 (0.036)	0.192 (.033)	.990 (.033)
Río Corobicí						
pooled	0.001 (.000)	0.998 (.009)	0.947* (0.010)	0.051 (0.009)	0.112 (0.000)	0.990 (0.055)
1997	0.000 (.000)	0.996 (0.015)	0.942* (0.018)	0.053 (0.013)	0.110 (0.004)	0.976 (0.053)
1998	0.000 (0.000)	0.984 (0.020)	0.924* (0.014)	0.060 (0.013)	0.109 (0.013)	0.990 (0.003)
1999	0.000 (0.000)	1.000 (0.018)	0.975* (0.010)	0.025 (0.011)	0.120 (0.012)	0.99 (0.012)
Río Tenorito (San Isidro)						
pooled	0.001 (0.000)	0.997 (0.013)	0.966* (0.009)	0.031 (0.009)	0.120 (0.001)	0.784 (0.105)
1997	0.002 (0.002)	0.950 (0.069)	0.932 (0.046)	0.018 (0.032)	0.165 (0.106)	0.911 (0.108)
1998	0.001 (0.000)	1.0 (0.000)	0.979* (0.005)	0.021 (0.005)	0.116 (0.000)	0.739 (0.073)

[1]Maternal inbreeding coefficient; [2]estimated multilocus outcrossing rate, * indicates estimate significantly different from t_m=0, *i.e.* estimated $t_m+2s_x<1.0$); [3]average of estimated single-locus outcrossing rate, * indicates estimate significantly different from t_m=0, *i.e.* estimated $t_s+2s_x<1.0$); [4]estimates biparental inbreeding, * indicates estimate significantly different from zero, *i.e.* $(t_m-t_s) > 2$.s.e); [5]estimated correlation of outcrossing rates, * indicates estimate significantly different from zero, *i.e.* r> 2.s.e.; [6]estimated correlation of outcrossed paternity

Table 7-18. Estimates of mating system parameters in seven population of *Plumeria rubra* located in forest fragments in northwestern Costa Rica (continued)

Population / year	$F\,(s_{\bar{x}})^1$	$t_m\,(s_{\bar{x}}.)^2$	$t_s\,(s_{\bar{x}})^3$	tm-ts $(s_{\bar{x}})^4$	$r_t\,(s_{\bar{x}})^5$	$r_p\,(s_{\bar{x}})^6$
Río Tenorito (Praderas)						
pooled	0.000 (0.000)	0.889 (0.092)	0.908* (0.041)	-0.019 (0.056)	0.179 (0.127)	0.990 (0.018)
1997	0.007 (0.010)	0.973 (0.139)	0.950 (0.065)	0.023 (0.086)	0.125 (0.037)	0.990 (0.029)
1998	0.000 (0.000)	0.879 (0.070)	0.893* (0.031)	-0.014 (0.041)	0.210 (0.001)	0.990 (0.001)
Río Magdalena						
pooled	0.000 (0.000)	0.914 (0.054)	0.901* (0.024)	0.013 (0.031)	0.125 (0.034)	0.964 (0.071)
1997	0.000 (0.000)	0.951 (0.039)	0.910* (0.023)	0.041 (0.019)	0.131 (0.034)	0.99 (0.007)
1998	0.000 (0.000)	0.730 (0.170)	0.859* (0.063)	-0.129 (0.073)	0.252 (0.194)	0.99 (0.023)
1999	0.002 (0.001)	0.988 (0.085)	0.945 (0.034)	0.043 (0.056)	0.105 (0.008)	0.593 (0.169)
La Pachanga						
1999	0.000 (0.000)	1.0 (0.003)	0.959* (0.015)	0.041 (0.015)	0.107 (0.001)	0.764 (0.106)
Sandillal						
pooled	0.013 (0.068)	0.999 (0.000)	0.960* (0.007)	0.040 (0.007)	0.103 (0.000)	0.82 (0.044)
1998	0.027 (0.122)	0.986* (0.006)	0.944* (0.012)	0.042 (0.007)	0.103 (0.004)	0.969 (0.019)
1999	0.013 (0.010)	1.0 (0.000)	0.974* (0.005)	0.026 (0.005)	0.103 (0.000)	0.646 (0.147)

[1] Maternal inbreeding coefficient; [2] estimated multilocus outcrossing rate, * indicates estimate significantly different from $t_m=0$, *i.e.* estimated $t_m+2s_x<1.0$); [3] average of estimated single-locus outcrossing rate, * indicates estimate significantly different from $t_m=0$, *i.e.* estimated $t_s+2s_x<1.0$); [4] estimates biparental inbreeding, * indicates estimate significantly different from zero, *i.e.* $(t_m-t_s) > 2$.s.e); [5] estimated correlation of outcrossing rates, * indicates estimate significantly different from zero, *i.e.* r> 2.s.e.; [6] estimated correlation of outcrossed paternity

Table 7-19. Linear regression of log of number of capsules tree^{-1} on dbh and neighbourhood density index of trees of *Plumeria rubra* located in six forest fragments in northwestern Costa Rica

Source	df	Mean square	F	R^2	significance[1]
Model	2	20.46	28.83		<0.0001
Error	73	0.71			
Variable	**df**	**parameter estimate and standard error**	**t**		
Intercept	1	1.470±0.2631	5.59		<0.0001
dbh	1	0.056±0.0079	7.12		<0.0001
density index	1	0.056±0.0253	2.22		0.0295

[1] *i.e.* probability of a higher value of 't' of 'F' under the null hypothesis

Table 7-20. Mean numbers of capsules tree^{-1} in trees of *Plumeria rubra* located in seven forest fragments in northwestern Costa Rica

Fragment	sample size	mean number of capsules tree^{-1} (standard error)
Quebrada Palmira	10	39.8±12.19
Corobicí	21	28.1±6.64
San Isidro	31	56.3±10.46
Praderas	19	24.0±4.74
Magdalena	20	23.0±5.39
Pachanga	6	43.8±12.45
Sandillal	4	98.0±25.64

Table 7-21. Analysis of covariance of effects of fragment and dbh on mean of log numbers of capsules tree^{-1} in six forest fragments of *Plumeria rubra* located in northwestern Costa Rica

Source	df	Mean square (Type III)	F	significance[1]
Fragment	5	1.0	1.47	0.21
dbh	1	29.2	40.71	<0.0001
Error	71	0.72		

[1] *i.e.* probability of a higher value of 'F' under the null hypothesis

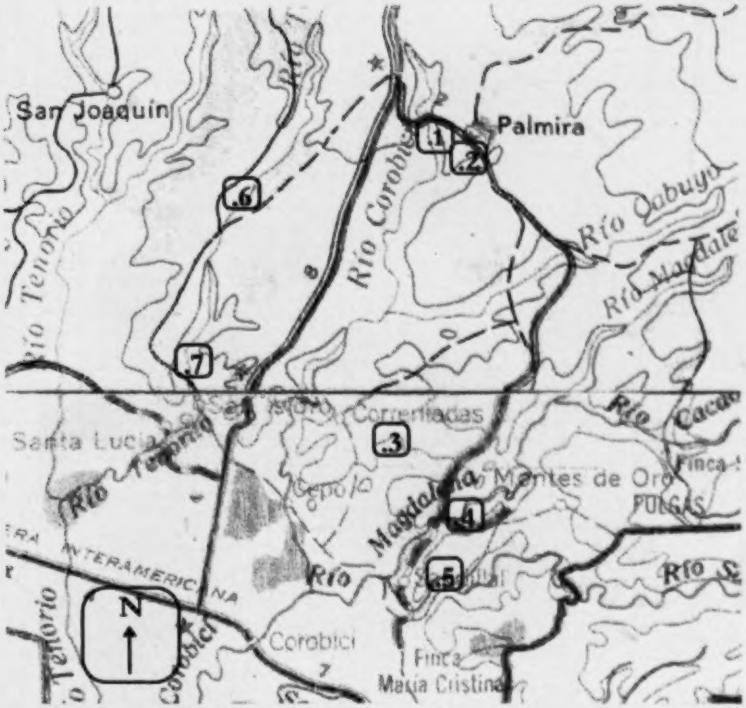
San Joaquín
Palmira
.1
.2
.6
Río Corobicí
Río Cabuyo
Río Magdalena
Río Tenorio
8
.7
San Isidro
Correntiadas
Santa Lucía
.3
Río Tenorio
Cepo
Montes de Oro
Finca
PULGAS
Río Cacao
.4
Magdalena
.5
Sandillal
Río
Río
INTERAMERICANA
N
Corobicí
Corobicí
Finca María Cristina
7
Tenorio

Figure 7-1. Populations sampled in a study of genetic effects of forest fragmentation on *Plumeria rubra* populations located near Cañas, Guanacaste province, Costa Rica. Periods mark population centres. Key to populations: 1: Río Corobicí; 2: Quebrada Palmira; 3: La Pachanga; 4: Magdalena; 5: Sandillal; 6: Praderas; 7: San Isidro; Scale: 1:130000. Shading (on original) indicates wooded areas, but not all such areas are included (source: IGN 1988a, 1988b, reproduced by permission, see Appendix 4).

```
                                                 + Q.Palmira
                                      +---------1
                                      !         +La Pachanga
                                   +--4
                                   !  !        +River Corobici
                                   !  +--------2
  +--------------------------------5           +River Magdalena
  !                                !
  !                                !       +---San Isidro
--6                                +-------3
  !                                        +---Praderas
  !
  +--------------------------------------------Sandillal
```

Figure 7-2. UPGMA dendrogram based on Nei's unbiased genetic distance between maternal trees in seven population of *P. rubra* located in northwestern Costa Rica.

```
                         +----------River Corobici
           +-------------1
  +--------2             +----------San Isidro
  !        !
--3        +------------------------Praderas
  !
  +---------------------------------Magdalena
```

Figure 7-3. UPGMA dendrogram based on Nei's unbiased genetic distance between four population of *P. rubra* located in northwestern Costa Rica (1997 progeny)

```
                                   +------Quebrada Palmira
                                +--1
               +----------------2  +------San Isidro
               !                !
  +------------4                +----------River Corobici
  !            !
--5            +---------------------------Praderas
  !
  !                          +---------------Magdalena
  +-------------------------3
                             +---------------Sandillal
```

Figure 7-4. UPGMA dendrogram based on Nei's unbiased genetic distance between four population of *P. rubra* located in northwestern Costa Rica (1998 progeny)

```
                                          +----Quebrada Palmira
                                      +---1
  +-----------------------------------2   +----River Corobici
  !                                   !
--4                                   +--------La Pachanga
  !
  !                                  +---------Magdalena
  +----------------------------------3
                                     +---------Sandillal
```

Figure 7-5. UPGMA dendrogram based on Nei's unbiased genetic distance between four population of *P. rubra* located in northwestern Costa Rica (1999 progeny)

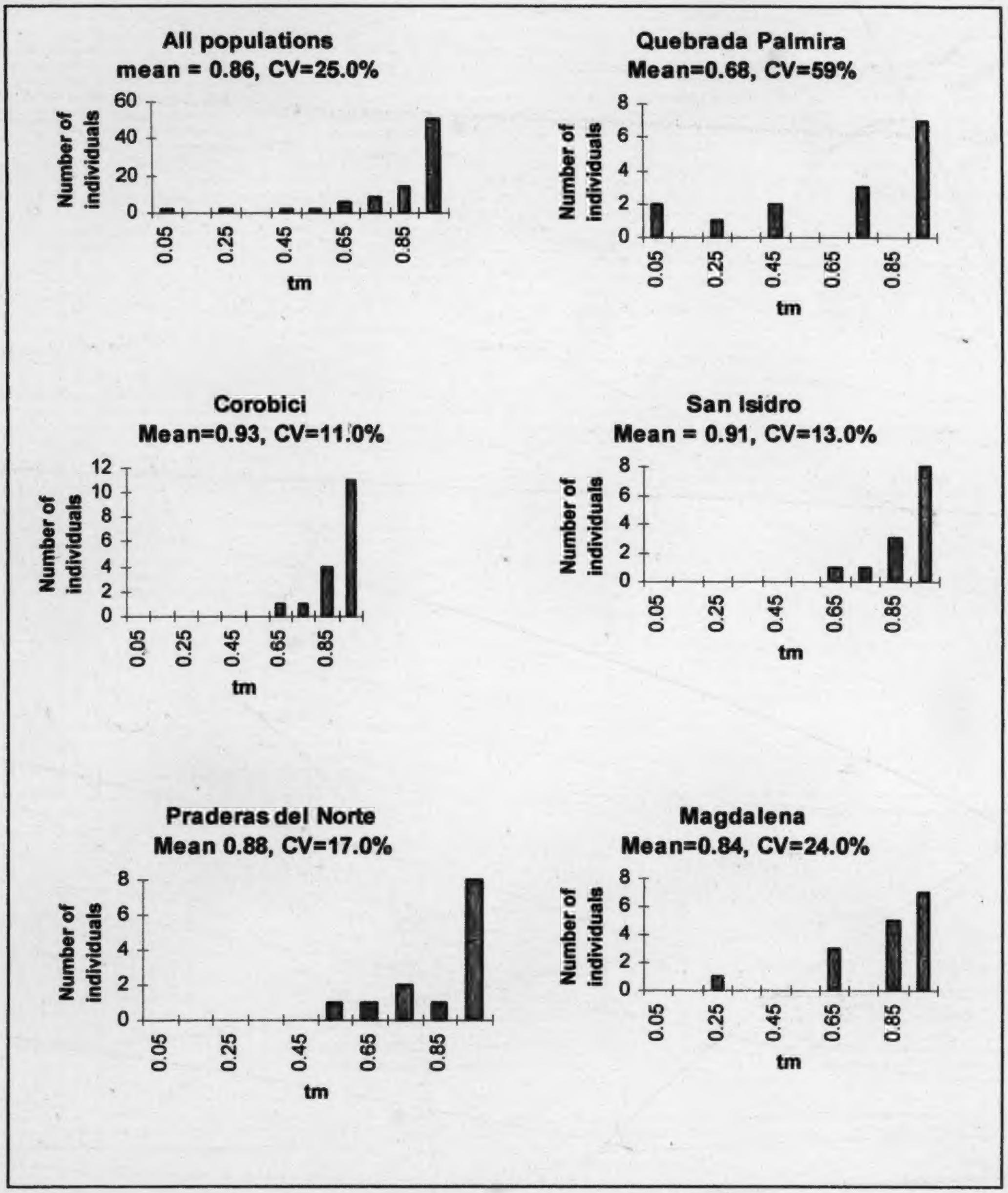

Figure 7-6. Frequency distributions of estimates of individual-tree outcrossing rates in seven populations of *Plumeria rubra* located in northwestern Costa Rica

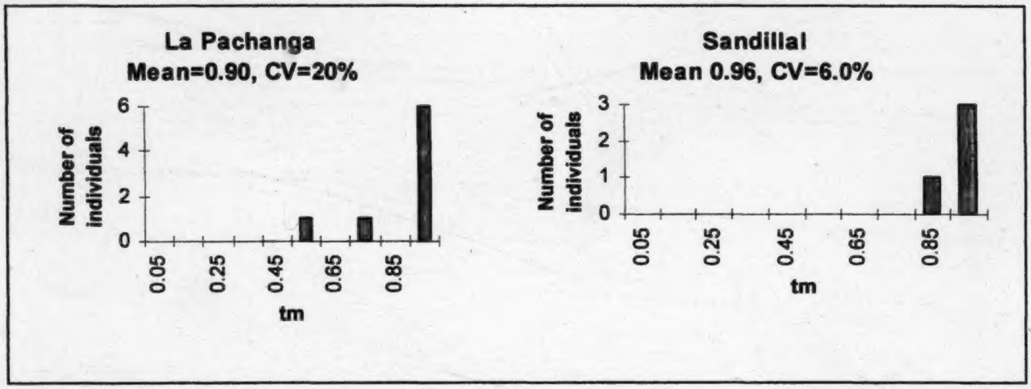

Figure 7-6. Frequency distributions of estimates of individual-tree outcrossing rates in seven populations of *Plumeria rubra* located in northwestern Costa Rica (continued)

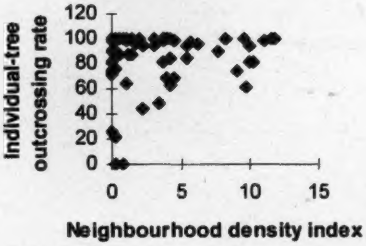

Figure 7-7. Scatter plot of relationship between neighbourhood density index and individual-tree outcrossing rate in trees of *Plumeria rubra* from 6 forest fragments in northwestern Costa Rica.

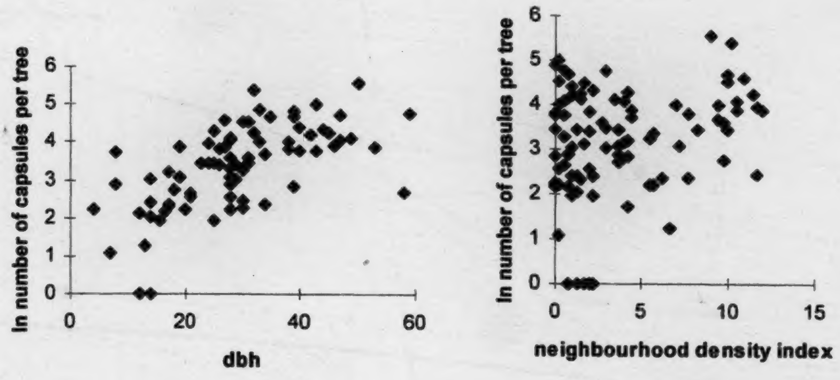

Figure 7-8. Scatter plots of log number of capsules tree-1 against dbh and neighbourhood density index rate in trees of *Plumeria rubra* from 6 forest fragments in northwestern Costa Rica.

Chapter 8

GENERAL DISCUSSION AND CONCLUSIONS: GENETIC IMPLICATIONS OF FOREST FRAGMENTATION FOR SPECIES OF LOWLAND GUANACASTE PROVINCE, COSTA RICA

INTRODUCTION

The foregoing chapters have documented variable but clear actual and incipient genetic impacts of forest fragmentation on both *Anacardium excelsum* and *Plumeria rubra*. In the case of *A. excelsum*, fragmentation and associated disturbance tends to lead to decline in census or effective population sizes due to reduced flowering, slower seedling growth rate, higher variance in fertility and lower outcrossing. *P. rubra* appears to be less prone to effects on outcrossing and fertility whilst, due to its highly mobile pollinator, it is likely to be less susceptible to genetic drift. However, even in this case, low genetic variation in one population (Sandillal), apparently caused by both founder effects and ongoing drift (due to restricted migration), coupled with apparent pollinator limitation of capsule production, illustrate that even in 'robust' species resilience to fragmentation is not unlimited. As indicated in the introduction (Chapter 1) to the present document, one of the justifications for individual species studies of this type is the generation of insights into the possible impact of fragmentation on other species. Accordingly, in this closing chapter this higher level of inference is considered, with particular reference to the tree species of the study zone itself. I begin with an overview of forest trees of the study zone, focussing particularly on those characteristics of particular relevance to the genetic effect of fragmentation, i.e. pollen and diaspore vectors, breeding systems and population density. Subsequently, in the light of these characteristics, I make some generalizations on the effect of fragmentation on outcrossing, variance in reproductive output, founder effects, gene flow and random genetic drift.

SPECIES OF THE STUDY ZONE

Jiménez *et al.* (1987) listed tree species found within the La Pacífica *hacienda*. Although some species present in the study zone are not found at La Pacífica, the list is sufficiently complete for present purposes. The following exotic species and possible partial domesticates were omitted from further consideration: *Mangifera indica*, *Spondias purpurea* (Anacardiaceae), *Thevetia peruviana* (Apocynaceae), *Jacaranda mimosifolia* (Bignoniaceae), *Bixa orellana* (Bixaceae), *Caesalpinia pulcherrima*, *Cassia fistula*, *Delonix regia*, (Caesalpinioideae), *Leucaena glauca*, *Tamarindus indica* (Mimosoideae), *Eucalyptus* spp., *Eugenia salamensis*, *Psidium* spp. (Myrtaceae), *Gliricia sepium* (Papilionioideae), *Citrus* spp. (Rutaceae), *Gmelina arborea*, *Tectona grandis* (Verbenaceae). One identification disputed by Janzen and Liesner (1980) (*Agonandra obtusifolia* (Opiliaceae)), identifications made by Jiménez *et al.* (1987) to generic level only (*Maytenus* spp. (Celastraceae), *Inga* spp. (Mimosoideae), *Ficus* spp. (Moraceae)) and Palmae were also omitted.

Breeding systems and compatibility

Species were classified according to breeding system and incompatibility, based, for the former, on the following sources: Area de Conservación Guanacaste (undated); Bawa and Opler (1975), Bawa *et al.* (1985a), Burger and Huft (1995); Center for Tropical Forest Science (undated); Croat (1979), Bullock (1985), Frankie *et al.* (1983), Haber and Frankie (1989), Janzen and Liesner (1980), Little and Wadsworth (1964), Little *et al.* (1974), Witsberger *et al.* (1982). For compatibility, classifications were based on Bawa (1974), Bawa *et al.* (1985b), Bullock (1985) and data from genetic markers (see Table A3-1 (Appendix 3)). Classifications are summarized in Table 8-1; details by species are included in Appendix Table A3-1).

The resulting classifications indicate that approximately 69 per cent of the listed species are hermaphroditic (Table 8-1). Of these, for those for which information exists, 74 per cent are self-incompatible, whilst the remaining 26 per cent show varying degrees of self-compatibility. When monoecious, androdioecious and gynodioecious species are included, 70.1 per cent of those for which compatibility data exist are self-incompatible. Approximately 18 per cent of the species are dioecious. As would be expected, the distribution of breeding systems is similar to that described by Bawa (1974) and Bawa and Opler (1975) for the COMELCO property, situated some 25km to the west. Bawa and Opler reported 20 per cent dioecy, and 79 per cent of (hermaphroditic) species as self-incompatible, a marginally higher proportion than reported here.

Pollen and diaspore vectors

Species were classed as pollinated by bats, hawkmoths, medium to large bees, hummingbirds, small insects (e.g. small bees, moths, butterflies), beetles and wind. Many of the classifications were made on the basis of published studies of the zone, particularly Heithaus *et al.* (1975), Haber and Frankie (1989) and Frankie *et al.* (1983). These and other specific sources are detailed in Appendix Table A3-2. Classifications unattributed to specific sources were based on floral morphology; in the great majority of cases, these were small-flowered species classed as pollinated by small insects. Classifications are summarized in Table 3-2; details by species are included in Appendix Table A3-2. Diaspore dispersal mechanisms were classified as either biotic or abiotic; the latter included species with no apparent dispersal mechanisms. Classifications unattributed to specific sources were based on fruit morphology, as noted in Table A3-2.

The classifications indicated that approximately 59 per cent of species have biotically dispersed diaspores (birds, bats, terrestrial mammals), whereas around 41 per cent are abiotically dispersed (mostly wind) (Table 8-2). These tendencies are consistent with the 53 per cent zoochory reported by Opler (1978, reproduced and cited in Bullock, 1995) for the Cañas area. Bats, hawkmoths and medium-to-large bees are the dominant pollinators for respectively 9.9, 11.1 and 26.3 per cent of the species, whereas small bees and other small insects account for 44 per cent of the total (Table 8-2).

Population density

Species population densities (number of trees ha^{-1}), based on data published by Glander and Nisbett's (1996) for riparian and 'upland' sites within La Pacífica, are detailed in Table A3-3. Species listed by Jiménez *et al.* (1987) but not registered in Glander and Nisbett's inventories were assigned zero densities in both site types. The mean number of stems $species^{-1}$ $hectare^{-1}$ for riparian and 'upland' (i.e. dry, non-riparian) sites at La Pacífica was $2.81ha^{-1}$ and $8.93ha^{-1}$, respectively (Table A3-3). The respective medians were $0.5\ ha^{-1}$ and $0.2ha^{-1}$; the divergence between the two measures reflects the presence of a number of species with high densities, particularly in the upland forest, which was dominated by *Lonchocarpus minimiflorus.*

Glander and Nisbett's data, although based on censi including all stems $\geq$1cm, are in general consistent with the expectation of low adult population densities for tropical dry forest trees (Hubbell, 1979), *i.e.* densities tend to be low even though the censi included size classes corresponding to juvenile plants. However, there are a number of species with relatively high population densities: *Guazuma ulmifolia*, *Lonchocaprus minimflorus*, *Myrospermum frutescens* (both site types), *Anacardium excelsum*, *Swietenia macrophylla* (riparian only), *Albizia caribaea*, *Cordia colococca*, *Cordia alliodora*, *Lysiloma divaricatum*, *Luehea candida*, *Trichilia americana*, *Casearia corymbosa*, *Machaerium biovulatum* and *Tabebuia ochraceae* (upland site) all have densities $\geq$10 stems ha^{-1}.

As reported in Chapter 3, these species subsist in the study zone as a whole in forest remnants of increasingly linear shape, interspersed with almost treeless agricultural land or relatively well (tree-)stocked pastureland. Morales and Kleinn (2001) report that the most common pastureland tree species in the Cañas area are (in order of frequency): *Guazuma ulmifolia*, *Enterolobium cyclocarpum*, *Pithecellobium saman*, *Tabebuia rosea*, *Cordia alliodora*, *Byrsonima crassifolia*, *Bombacopsis quinata*, *Acrocomia vinifera*, *Mangifera indica*, *Cedrela odorata*, *Tabebuia ochraceae*, *Dalbergia retusa*, *Spondias mombin*, *Chione costaricensis*, *Albizia caribaea*, *Cassia grandis* and *Acosmium panamense.*

Clearly, species of the study zone are characterized by a wide range of reproductive and other characteristics, each the product of many generations of evolutionary change, in which adaptive response to natural selection may be assumed to have played an important role. The impact of fragmentation on genetics of these species is likely to depend on the population genetic implications of these characteristics, given that, whilst 'abapted' (Begon *et al.*, 1990) by past environments (*i.e.* evolved by natural selection under past, rather than present, conditions), these must 'perform' in present-day, human-modifed environments. Obviously, the implications of fragmentation for each species cannot be identified without detailed, individual studies. However, given the findings of the present study, the species characteristics outlined above, and *a priori* considerations, some general points may be made and some specific examples cited.

EFFECTS ON OUTCROSSING

The findings for *A. excelsum*, in conjunction with previous studies (Karron *et al*, 1995; Ghazoul *et al.*, 1998; Murawski and Hamrick, 1992; Murawski *et al.* 1990; Prober and Brown 1994) appear to confirm the potential of fragmentation-mediated reductions in tree density to lead to increased selfing rates. This would primarily affect self-compatible species, such as *Ardisia revoluta*, *A. excelsum*, *Ceiba pentandra*, *Curatella americana*, *Calycophyllum candidissimum*, *Malpighia glabra*, *Muntingia calabura*, *Prockia crucis*, *Sloanea terniflora*, although individual trees of generally otherwise self-incompatible species may also be self-compatible (see Bawa, 1974). As the effect results from changes in pollinator behaviour, it seems likely to be strongest in the case of species with relatively weak-flying pollinators, i.e., from the above list, *A. revoluta*, *A. excelsum*, *C.candidissimum*, *P. crucis*, *S. terniflora* (see Table A3-2). As outlined in connection with *A. excelsum* (Chapter 5) density reduction may occur with habitat degradation, or because of increased linearization of habitat (as a consequence of edge effects). Particularly in the case of species with lower population densities than *A. excelsum*, fragmentation may also lead directly to increases in nearest neighbour distance, i.e. through removal of matrix conspecifics. Although reduced tree density cannot generally lead to increased selfing in self-incompatible species, it should be noted that it may nevertheless be associated with increased geitonogamous pollination (without fertilization). It follows that even in such species, decreased tree density may negatively affect tree fertility.

In extreme cases of fragmentation, self-compatibility offers clear advantages, as pointed out by Baker (1955) in the context of colonization. Evidently, for $N=1$ and restricted migration, a self-compatible species has a higher chance of persistence in a given fragment than a self-incompatible or dioecious species. Given the low population density of many species of the zone, the capacity for self-fertilization may be an important influence in determining future species composition of isolated fragments.

HIGH VARIANCE IN REPRODUCTIVE OUTPUT

High variance in reproductive output, as noted in the case of some *A. excelsum* populations, is likely to result from two causes, which may be termed negative and positive effects. The negative case, in which normal levels of fertility are depressed in all but a few individuals (as in many *A. excelsum* fragments in the present study) is most likely to occur in species susceptible to fragmentation-induced resource limitations. Many mesophytic species in highly disturbed fragments may be subject to such effects, e.g. *Ardisia revoluta, Andira inermis, Inga vera, Licania arborea, Ocotea veraguensis, Pithecellobium longifolium, Terminalia oblonga.* The positive case is exemplified by Aldrich and Hamrick's (1998) discovery of reproductive dominance of pastureland trees in *Symphonia globulifera.* Several of the common pastureland species listed by Morales and Kleinn (2001) also occur in riparian and other forest fragments, e.g. *G. ulmifolia, E. cyclocarpum, Pithecellobium saman, Tabebuia rosea, T. ochraceae, B. quinata.* Given the large size of many of the pastureland trees, particularly *E. cyclocarpum* and *P. saman*, there is potential for 'secondary bottlenecks' similar to those described by Aldrich and Hamrick for *S. globulifera.* However, with the exception of *Byrsonima crassifolia,* itself unusual in forest fragments in the study zone, none of the common pastureland species listed by Morales and Kleinn are self-compatible, thus reducing the risk of the 'swamping' of remnants with selfed propagules of highly fertile pastureland trees, as observed by Aldrich and Hamrick (1998).

FOUNDER EFFECTS

Low population densities imply acute founder effects, particularly in smaller fragments. Hubbell (1979) found that distribution of dry forest tree species tended to be clumped rather than evenly or randomly distributed. In a sense, clumping might mitigate founder effects, in that, whilst reducing the chance of species representation in any given fragment, at the same time it implies that more than one individual may be found in surviving fragments. However, single or very few individuals neverthless occur in many small fragments. For example, the Toronja fragment (area approximately 1ha) (see Chapter 5) contains one individual of *Spondias mombin* (Anacardiaceae) and two individuals of the endangered 'cannonball-tree', *Couroupita nicaraguensis* (Lecythidaceae). Similarly, the Ojoche fragment contains one *Brosimum alicastrum* ('ojoche') (Moraceae) tree. Furthermore, clumps of individual species may frequently be the product of limited seed dispersal rather than, necessarily, clumped habitat, and may therefore be more highly related than would be expected by chance. For example, the Bosque Duquesa fragment contains a small group of trees of the wind-dispersed bombacaceous species *Pseudobombax septenatum*, which is rare in similar habitat throughout the study zone. Such relatedness within clumps will tend to increase founder effects relative to expectations based on population sizes alone.

GENE FLOW AND RANDOM GENETIC DRIFT

Given the combination of low population densities and clumping of conspecifics, the capacity for at least occasional long-distance dispersal of gametes is likely to be of adaptive value even in undisturbed tropical dry forest. However, fragmentation will, in many cases, lead to a more extreme degree of population aggregation than under natural conditions, and it is possible that under such changed conditions some species's capacity for long-term dispersal may fall short of that consistent with maintenance of adequate genetic diversity in fragmented landscapes. The respective findings for *A. excelsum* and *P. rubra* illustrate this point. Within the Corobicí group, *A. excelsum* shows notably higher subpopulation differentiation, with F_{st} values approximately three times those of *P. rubra* (disregarding the atypical Sandillal population), even though the area covered by the *P. rubra* populations is actually somewhat larger than that of the *A. excelsum* populations. *P. rubra* is a more naturally-fragmented and less abundant species than *A. excelsum*. *A priori*, it might be expected to exhibit lower levels of genetic diversity and higher levels of population subdivision. That this is not the case seems likely to be due at least in part to the greater mobility of *P. rubra*'s hawkmoth pollinator. The pollination system appears to some degree to preadapt the species to forest fragmentation: *i.e.*, the same factor that permits its persistence in naturally fragmented populations equips it also to persist within a fragmented forest set in a deforested matrix.

In Table 8-3, the data presented in Table 8-2 is further reduced to two pollen vector classes: relatively long-distance vectors, i.e. bats (Bawa, 1990), hawkmoths (Haber and Frankie, 1989), medium to large bees (Janzen, 1971; Frankie *et al.*, 1976), fig wasps (Nason and Hamrick, 1997) and wind, and relatively short distance (small bees, beetles, small diverse insects) (Bawa and Opler, 1975). As, in general, biotic diaspore vectors are expected to be associated with more frequent long distance dispersal, the resulting cross-classification yields four (gametic) dispersal groups, which may be broadly characterized as 'long-long' (LL; i.e. biotic diaspore vector, long-distance pollinator), LS, SL and SS. This two by two classification of seed and pollen dispersal vectors reveals significant non-independence between the two factors (p=0.007, Fisher's exact test), with higher than expected numbers in each of the two LS/SL categories, and lower than expected numbers in LL and SS (Table 8-3). That is, the implication is that even in unfragmented conditions (*i.e.* those under which the mechanisms in question evolved), for persistence of most species it has been advantageous to have at least one (and perhaps not more than one) dispersal mechanism more suited to long-distance dispersal, and only relatively rarely has the absence of both long-distance dispersal mechanisms not been disadvantageous (only approximately 11% of the species fell in the SS dispersal group).

P. rubra and *A. excelsum* both fall in the intermediate, 'long-short' (*i.e.*, LS or SL) dispersal categories. Even in these cases, there are several strands of evidence that gene flow correlates with distance: firstly, the presence of correlation between genetic and geographic distance in both species; secondly, the correlation between outcrossing rate and tree density in *A. excelsum*; thirdly, correlation between fruit production and tree density in *P. rubra*; fourthly, the low genetic variation present in the Sandillal population of *P. rubra*. It might be speculated that, in undisturbed forest, species with neither long distance pollinators nor highly mobile diaspore vectors tend to form relatively large continuous stands or may be habitat generalists, i.e. with less clumped natural distributions. For species of both such groups (*e.g.* the dioecious Anacardiaceae *Astronium graveolens*, which is wind-dispersed, apparently pollinated by small insects, and found in both riparian and dry conditions) fragmentation may imply a very much more aggregated population distribution, and the SS dispersal syndrome may no longer be capable of maintaining adequate levels of inter-population gene flow. It seems reasonable to suggest that species without either highly mobile pollen or diaspore vectors will tend to be less able than either study species to maintain gene flow in fragmented landscapes. On the basis of gene flow considerations alone, the following species would all appear to be at greater risk of fragmentation effects than either of the two study species: *Alvaradoa amorphoides*, *Acosmium panamense*, *Astronium graveolens*, *Calcyophyllum candidissimum*, *Cedrela odorata*, *Cordia alliodora*, *Esenbeckia litoralis*, *Lonchocarpus minimiflorus*, *Maechaerium bivulatum*, *Swietenia macrophylla*, *Thouinidium decandrum*. The presence of trees in intervening pastureland matrices would tend to reduce risk. Of the above species, three (*Acosmium panamense*, *Cordia alliodora*, *Cedrela odorata*) are common in pastureland in the Cañas area (Morales and Kleinn, 2001). However, at present, pastureland trees, which are generally remnants of forests or traditional grazing *sitios*, appear not to be being replaced. Although their final disappearance may take many decades, their eventual loss seems inevitable under current farming practice. It follows that their contribution to maintenance of gene flow may be transient.

The classification of dispersal categories in Table 8-3 also assumes that dispersal vectors are actually present. This may not always be the case. Janzen and Martin (1982) argued that seeds of many tree species of lowland Guanacaste (e.g. *Crescentia alata*, *Enterolobium cyclocarpum*, *Hymenaea courbaril*, *Pithecolbium saman*) were formerly dispersed by the extinct Central American Pleistocene megafauna. Although their argument has been strongly criticized by Howe (1985), the latter author nevertheless concedes its possible applicability to particular species (i.e. those with 'botanical anomalies' such as indehiscent, pulp-filled fruit). Particularly in the case of seed that is never or rarely eaten by birds, bats or monkeys, this implies truncation of present-day seed shadows, i.e. relative to pre-extinction shadows. Although Janzen and Martin (1982) point out the role of cattle and horses in substituting for extinct species, these are not always present. Even in pastureland matrices, they may be fenced out of forest fragments, particularly riparian fragments. Furthermore, it is necessary to distinguish dispersal in general from long-distance dispersal. The Pleistocene megafauna, like the semi-feral cattle herds of colonial Costa Rica (see Chapter Three), could wander and graze over vast areas. By contrast, under modern stock management practices, cattle are regularly moved between relatively small enclosed pastures. The largest current pastures rarely exceed 20 ha (Francisco Mesén, personal communication). In a roughly square pasture of this size, seed dispersal would be limited to approximately 500m, although occasional longer distance dispersal could occur if seed ingested in one pasture is defecated after rotation of cattle to a different pasture.

Ultimately, the impact of fragmentation depends not only on its interaction with biological characteristics and processes but also on what might be done in mitigation of negative effects. Logically, such mitigation implies reversal or halting of those processes likely to lead to genetic erosion. In the agricultural and pastoral landscapes that, outside protected areas, characterize much of Pacific Central America, it seems likely that such reversal is most likely to be achieved by reinforcing and stimulating 'positive' tendencies already present, *i.e.* as seen in the partial retention, described here, of riparian 'buffer strips' and pastureland trees.

In the case of riparian forest, some further destruction could be prevented by effective application of existing forest law, which has been clearly flouted in parts of the study zone. There is increasing awareness of such issues in Costa Rica (e.g. Campos *et al.*, 2001), and increasing political will to deal with the problem (*e.g.* one of the authors of the latter publication is the current Costa Rican Minister of Environment; see also Rodríguez, 2003). However, willingness to prevent further clearance of riparian forest will not restore that which has already been lost.

Both riparian strips and pastureland trees are potential or actual providers of a range of goods and services, which offer many potential or actual economic and financial benefits. In the case of riparian forest, these include, for example, maintenance of water quality, regulation of streamflow, streambank protection, biodiversity protection, tourism (e.g. in the study zone at least two companies offer rafting trips down the River Corobicí; these would be of little interest without the riverside forest), fuelwood, fruits, game, shelterbelt functions, shade for river-users (e.g. fishermen, bathers, clothes-washers), aesthetics, medicinal plants, timber, dry-season grazing (as still practised in the study zone, e.g. in Bosque Duquesa and Quebrada Reventado). In the case of pastureland trees, these include shade and dry season fodder for cattle (e.g. fruits of genízaro (*Pithecellobium saman*) (Durr, 2001) or guácimo (*Guazuma ulmifolia*) (Casasola, 2000), timber, fence-posts (Harvey and Haber, 1999), and many of those mentioned in connection with riparian strips, all within the context of an alternative land-use (i.e. silvopastoral systems) which may offer multiple advantages over now-traditional, monoculture-based pastureland systems (Sánchez, 2002).

The case for active restoration or expansion of riparian strips, together with promotion of increasing use of pastureland trees, needs to be made on the basis of economic and financial valuation of these goods and services within the framework of economic and financial analyses of the production systems in question. The compatibility of alternative uses would also need to be considered. Presuming that systems with positive net benefits, in broad economic or narrower financial terms, can be identified, a number of options are available for stimulating and facilitating their uptake. Payment for environmental services, a scheme for which is already in place in Costa Rica, represents one option to secure non-market benefits, particularly for expansion of riparian strips beyond widths required by law. If compatible 'productive' (e.g.

grazing, harvesting of non-traditional products) uses of the same forest could be identified, this would further increase the attractiveness of riparian strip conservation to landowners. In the case of silvopastoral systems, Ibrahim *et al.* (2002) identify adequate dissemination, identification of farmer preferences and provision of sources of capital (*e.g.* through credit) as key elements in adoption of systems. For both, a key component in wider adoption would be active and genuine (*q.v.* Enters, 2000) stakeholder participation in identification and valuation of goods and services, evaluation of systems and pilot project design.

Sánchez-Azofeifa *et al.* (2001) remark that 'the current conservation strategy in Costa Rica may only preserve a small fraction of nature's wealth. More attention must be given to ecosystem restoration and regeneration in highly degraded tropical environments'. Given the long-distance gene flow that has been observed in a number of species (Apsit *et al.*, 2001; Dick, 2001; White and Boshier, 2000; Nason and Hamrick, 1997), the improved conservation and restoration of riparian fragments, coupled with more active husbandry of pastureland trees, could make an important contribution to both maintaining biodiversity outside protected areas and ensuring reproductive connectivity between them. At present, such measures are clearly not in place, and the impact of forest fragmentation will depend largely on the biological characteristics of species in the prevailing environmental conditions. Inaction is likely to lead to gradual erosion of species richness of remaining fragments, coupled with increased isolation and genetic erosion of remaining populations of both rare and more common species.

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Table 8-1. Summary of breeding systems and self-compatibility of 114 tree species of Hacienda La Pacífica, Cañas, Guanacaste province, Costa Rica[1]

	Incompatibility			
Sexual System	Self-incompatible	Self-compatible	No data	Totals
Hermaphroditic	26	9	44	79
Monoecious	7	0	4	11
Andromonoecious	1	1	0	2
Gynodioecious	0	1	0	1
Subtotals	34	11	48	93
Dioecious	21	n.a.	n.a.	21
TOTALS	89	11	48	114

[1] Source: see Appendix 3, Table A3-1

Table 8-2. Summary of pollen and diaspore vectors of 114 tree species of Hacienda La Pacífica, Cañas, Guanacaste province, Costa Rica[1]

	Diaspore vector			
Pollen vector	Biotic	Abiotic or no apparent dispersal mechanism	Insufficient data	Totals
bats	4	5	0	9
hawkmoths	4	7	0	11
medium-to-large bees	11	14	1	26
wind	2	0	0	2
fig-wasps	4	0	0	4
Subtotal	25 (23)[2]	26	1	52
hummingbirds	2	0	0	2
moths, small bees, diverse small insects	33	11	0	44
Beetles	3	0	0	3
Subtotal	36	11	0	47
insufficient data	3	7	5	15
Totals	66(64)	44	6	116(114)[2]

[1] Source: see Appendix 3, Table A3-2 [2] figures in parentheses are totals after adjusting for repetition of *Inga vera* and *Bombacopsis quinata*, both of which appear as both hawkmoth and bat pollinated

Table 8-3. Two by two classification of pollen and diaspore dispersal vectors of 98 forest tree species in Cañas, Guanacaste province, Costa Rica[1]

	Diaspore vector		
Pollen vector	Bioti[illegible]	Abiotic	Totals
	observed (expected) numbers of species by class		
Long-distance[2]	25 (31.7)	26 (19.3)	51
Short-distance[3]	36 (29.3)	11 (17.7)	47
Totals	61	37	98

[1] Source: see Appendix 3, Table A3-3 [2] bats, hawkmoths, medium to large bees, fig-wasps, wind; [3] moths, small bees, small diverse insects, beetles. Hummingbirds not considered long-distance pollinators because of lack of information on territoriality of the species in question (Bawa, 1990); territoriality could lead to low expected pollen movement even for strong flyers

APPENDICES

Appendix One

Details of laboratory procedures

ANACARDIUM EXCELSUM

Enzyme extraction

Samples were prepared in batches of 20. Fresh, healthy leaves were cut with scissors from seedlings in the greenhouse, placed in plastic ziplock bags and taken immediately to the laboratory. During final preparation of sample tubes in the laboratory, the leaves were held for appoximately five minutes at 4°C. From each leaf, 4 sample disks were cut using the lid of an 1.5ml Eppendorf tube, for an approximate area of $2cm^2$ (for tough leaves, an equivalent area was cut with scissors; for ease of preparation, succulent leaves were used whenever possible). The 20 samples were then placed on ice in 1.5ml Eppendorf microcentrifuge tubes. For grinding, each tube with its contained sample was dipped momentarily in liquid nitrogen, using tongs. When the nitrogen had almost evaporated, the sample was crushed and ground for around five seconds using plastic pestles. Subsequently, 120μl of Liengsiri *et al.*'s (1990) extraction buffer #9 (Tris-HCl pH 8.0 0.1M, Ascorbic acid 0.01M, Cysteine HCl 5.4mM, $MgCl_2$ 0.2%, $CaCl_2$ 0.2%, PEG 20M 1%, Sucrose 0.5M, Tween 80 1%, Tergitol 1%, ß-mercaptoethanol 0.3%, pH adjusted with 1M tris) was added to the tube using a Gilson pipette. Grinding was continued until the extraction buffer melted and a thick slurry was obtained. The tube was then capped and placed back on ice. After centrifugation (10000 rpm, 10 minutes, 4°C), two filter paper (Whatman #3) wicks, each 2.5x8.0mm, were dipped in each sample extract and then placed in a different Eppendorf tube. The tubes with wicks were then packed in ice in ziplock brand plastic containers and placed at -80°C until needed.

Gel preparation

The pH5.7 histidine-citrate buffer system described by Wendel and Weeden (System 1) was used. The electrode buffer of this system has molarities 0.065 (histidine free base) and 0.019M, (citric acid, monohydrate), whilst the gel buffer is prepared as a 1:6 dilution of the electrode buffer. According to Wendel and Weeden, this results in 0.009M molarity histidine (i.e. 0.065/7) and 0.006M citric acid. However, the latter molarity does not correspond to the dilution ratio, which implies final molarity of 0.019/7 = 0.0027M. In the preparation of the gel buffer, the dilution ratio specified by Wendel and Weeden was retained, i.e. molarities used were 0.009M histidine and 0.0027M citric acid.

For preparation of each 12.5% starch gel, 170ml of the gel buffer was placed in a 1*l* Ehrlenmeyer flask and heated for 2 minutes and 40 seconds in a microwave oven (Magic Chef, 850W), whilst 110ml of the buffer was mixed with 35g of hydrolized potato starch (Connaught Laboratories, Ontario) in a 1*l* vacuum flask. The boiling buffer was then poured into the starch suspension, and shaken vigourously for about 4 seconds. It was then returned to the microwave for a further 2 minutes and 10 seconds (i.e. until 'glassy'), removing after 30, 60 and 90 seconds in order to swirl the mixture, for greater uniformity. After removal, the mixture was degassed using a Nalgene tap-attached aspirator pump for approximately 25 seconds, and then poured into a previously assembled gel mould with internal dimensions 8x22x1cm. After 1 hour, the gel was covered with cling film and cooled to 4°C before use (when poured in the evening for use the next morning, the mould with gel was stored in a ziplock bag in the dark at room temperature).

Gel loading and running

Gels were loaded on ice with the previously prepared wicks, and run initially for 15-20 minutes at 25mA on one of the following power units: Bio-Rad Model 250/2.5 Power Supply, E-C Apparatus Corporation VWR 105, E-C Apparatus Corportaion EC105, Gelman Sciences Inc. Deluxe Regulated Power Supply. The gels were then dewicked, and current raised to 50mA. Gels were then run for 4 hours after dewicking. As gels were run in a 25°C laboratory, ziplock bags with crushed ice were placed on each gel and changed every 15 minutes.

Staining

On termination of the run, each gel was sliced into 1mm or 2mm slices using monofilament sewing thread and 1mm thick plastic guide strips. The slices were then transferred to staining trays (Rubbermaid stackable storage trays, 22.5x7.5cm), and stained using the following buffers (quantities given are for one gel slice).

Adenylate kinase

Tris HCl 0.2M, pH8.0: 7.5ml
ADP: 80mg

Glucose: 90mg
G6PDH: 40 units
Hexokinase: 264 units
1% BSA: 0.5ml
10% $MgCl_2$: 0.5ml
1% MTT: 1ml
1% NADP: 1ml
1% PMS: 0.25ml
After mixing, the stain buffer was combined with 12.5*ml* 2% agarose. The latter had been prepared previously and held in a 60*℃* waterbath until needed.
Source: adapted from Liengsiri *et al.* (1990) and Wendel and Weeden (1990).

Phosphogluconate dehydrogenase

Tris HCl 0.2M, pH 8.0: 5ml
Phosphogluconic acid, barium salt: 15mg
1% $MgCl_2$: 0.5ml
1% MTT: 1ml
1% NADP: 1ml
1% PMS: 0.25ml
After mixing, he stain buffer was combined with 12.5ml 2% agarose. The latter has been prepared previously and held in a 60°C waterbath until needed.
Source: Liengsiri *et al.*,1990.

UTP-glucose-1-phosphate uridylyltransferase

Tris HCl 0.2M, pH 8.0: 12.5ml
Tetrasodium pyrophosphate: 10mg
Phosphogluconic acid, barium salt: 15mg
UGDP: 25mg
1% BSA: 0.5ml
PGM: 50 units
G6PDH: 40 units
1% $MgCl_2$: 0.5ml
1% MTT: 0.5ml
1% NADP: 0.5ml
1% PMS: 0.25ml
After mixing, the stain buffer was combined with 12.5ml 2% agarose. The latter has been prepared previously and held in a 60°C waterbath until needed.
Source: Hodgkiss, 2001

Phosphoglucomutase

Tris HCl 0.2M, pH 8.0: 25ml
Glucose-1-phosphate: 250mg
G6PDH: 25 units
1% BSA: 0.5ml
1% $MgCl_2$: 0.5ml
1% MTT: 1.0ml
1% NADP: 1.0ml
1% PMS: 0.25ml
Source: adapted from Hodgkiss (1991), Liengsiri *et al.*, 1990.

Malate dehydrogenase

Tris HCl 0.2M, pH 8.0: 25ml
DL-Malic acid 0.5M pH7.0: 2ml
1% MTT: 0.5ml
1% NAD: 0.5ml
1% PMS: 0.25ml
Source: adapted from Liengsiri *et al.*, 1990.

Leucine aminopeptidase

LAP stain buffer, pH6.0 (1:1 0.2M Tris HCl pH8.0, 0.2M Maleic anhydride): 25ml
4% L-leucine ß-napthyl-amide: 2ml
Fast K salt: 25mg in 2ml N,N-dimethylformamide
10% $MgCl_2$: 0.5ml
Source: adapted from Liengsiri *et al.*, 1990, Wendel and Weeden, 1990.

After adding the stain buffers, the gels were incubated for 1 hour (MDH) or 2 hours (others) at 37°C. After removal from the incubator, LAP gels were rinsed in 50% glycerol before photographing.

PLUMERIA RUBRA

Enzyme extraction

Seeds of each pod-within-family were germinated on moistened chromotography paper. One to two days after germination, radicle tips of approximately 0.5cm length were cut using a scalpel (samples were prepared in batches of 20). The tips were transferred to prelabelled 1.5ml Eppendorf microcentrifuge tubes held on ice. Subsequently, 30µl of Liengsiri *et al.*'s (1990) extraction buffer #9 (Tris-HCl pH 8.0 0.1M, Ascorbic acid 0.01M, Cysteine HCl 5.4mM, $MgCl_2$ 0.2%, $CaCl_2$ 0.2%, PEG 20M 1%, Sucrose 0.5M, Tween 80 1%, Tergitol 1%, ß-mercaptoethanol 0.3%, pH adjusted with 1M tris) was added to the tube using a Gilson pipette and the sample was crushed and ground briefly (10-15 seconds) using a plastic pestle. Two filter paper (Whatman #3) wicks, each 2.5x8.0*mm*, were dipped in each sample extract and then placed in a different Eppendorf tube. The tubes with wicks were then packed in ice in ziplock brand plastic containers and placed at -80*°C* until needed.

Gel preparation

Two running buffer systems were used: Pitel and Cheliak's (1984) pH 7.0 Histidine system (electrode buffer: 0.125M Tris, pH adjusted to 7.0 with 1M citric acid; gel buffer: Histidine HCl 0.05M, EDTA 1.4mM, pH adjusted to 7.0 with 1M Tris) and Ridgeway *et al.*'s (1970) lithium borate / tris citrate system (electrode buffer: lithium hydoxide 0.06M, boric acid 0.3M; Tris 0.03M, citric acid 0.005M, 1% electrode buffer, pH adjusted to 8.5 with 1N NaOH). The 12.5% starch gels were prepared as for *A. excelsum* (see above).

Gel loading and running

Gels were loaded on ice with the previously prepared wicks, and run initially for 15-20 minutes at 70V and 100V for respectively the histidine and lithium borate systems, using one of the following power units: Bio-Rad Model 250/2.5 Power Supply, E-C Apparatus Corporation VWR 105, E-C Apparatus Corportaion EC105, Gelman Sciences Inc. Deluxe Regulated Power Supply. The gels were then dewicked, and voltage doubled. Gels were then run for 4 hours after dewicking. The gels for the 1998 and 1999 collections run in a 25°C laboratory; ziplock bags with crushed ice were placed on each gel and changed every 15 minutes. Gels for the 1997 collection were run in a 4°C cold room. In this case, icepacks were changed every hour.

Staining

On termination of the run, each gel was sliced into 2mm slices using monofilament sewing thread and 1mm thick plastic guide strips. The slices were then transferred to staining trays, and stained using the following buffers (quantities given are for one gel slice):

Histidine system

Alcohol dehydrogenase

0.2M Tris-HCl, pH 8.0: 25ml
95% ethanol: 5ml

1% NAD: 0.5ml
1% NBT: 0.5ml
0.5% PMS: 0.5ml
Source: Liengsiri *et al.*, 1990

Phosphoglucomutase

Tris HCl 0.2M, pH 8.0: 25ml
Glucose-1-phosphate, disodium salt: 250mg
G6PDH: 25 units
1% $MgCl_2$: 0.5ml
1% MTT: 0.5ml
1% NADP: 0.5ml
0.5% PMS: 0.5ml
Source: adapted from Liengsiri *et al.*, 1990, Wendel and Weeden, 1990.

Phosphogluconate dehydrogenase

Tris HCl 0.2M, pH 8.0: 5ml
Phosphogluconic acid, barium salt: 15mg
1% $MgCl_2$: 1ml
1% MTT: 1ml
1% NADP: 1ml
0.5% PMS: 0.5ml
Source: Liengsiri *et al.*, 1990

lithium borate / tris citrate system:

Aspartate aminotransferase

Pyridoxal-5-phosphate: 2mg
Fast Blue BB salt: 50mg
AAT substrate solution: 25ml
(For 500ml of AAT substrate solution: dissolve 2.65g L-aspartic acid, 0.35g L-ketoglutaric acid in about 300ml of 0.2M Tris-HCl, adjust to pH 8.0 with 1N NaOH and top up to 500m with 0.2M Tris-HCl pH 8.0).
Source: Liengsiri *et al.*, 1990

Glucose-phosphate isomerase

Tris HCl 0.2M, pH 8.0:
Fructose-6-phosphate, disodium salt: 12.5mg
Glucose-6-phosphate dehydrogenase: 5 units
1% $MgCl_2$: 0.5ml
1% MTT: 0.5ml
1% NADP: 0.5ml
0.5% PMS: 0.5ml
Source: Liengsiri *et al.*, 1990

After adding the stain buffers, the gels were incubated for 1 hour at 37°C.

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Appendix Two

Modified maternal genotypes of *Plumeria rubra*

As explained in Chapter 7, some inferred maternal genotypes of *P. rubra* were reassigned based on capsular segregation ratios. These are detailed below (Table A2-1).

Table A2-1. Reassigned maternal inferred genotypes of *Plumeria rubra.*

Population	Family	Inferred maternal multilocus genotype	Modified maternal multilocus genotype	Justification for modification
Quebrada Palmira	Cemetery tree	BBABAAABAB	BBABAAAAAB	Ratios of AA:AB progeny in pods 563 (7:2), pods 564 (6:1).
Río Corobicí	1000	ABABAAAAAB	AAABAAAAAB	Ratios of AA:AB progeny in pod 139 (6:0).
	1001	ABBBAAABAB	ABBBAAAAAB	Ratios of AA:AB progeny in pods 19 (4:1), 20(7:0), 21 (8:0).
	1002	ACBBAAABAA	ACBBAAAAAA	Ratios of AA:AB progeny in pod 506 (6:0).
	1004	ACBBAAABAB	AABBAAABAB	Ratios of AA:AC progeny in pods 147 (5:0), 513 (4:0), 515 (6:0).
	1005	AAABAAACAB	AAABAAAAAB	Ratios of AA:AC progeny in pods 518 (4:0), 520 (6:0).
	1010	AAABABACAB	AAABABACAB	Ratios of AA:AB progeny in pod 169 (19:0).
	1011	ABABAAAAAB	ABABAAAAAB	Ratios of AA:AB progeny in pods 6 (6:0), 550 (7:0).
	1021	ABABAAAAAB	ABBBAAAAAB	Ratio of BB:AB progeny in pod 180 (13:0).
Magdalena	4010	ABBBABAAAB	ABBBAAAAAB	Ratio of AA:AB progeny in pod 73 (12:0).
La Pachanga	CS04	ABABAAAABB	AAABAAAABB	Ratio of AA:AB progeny in pod 476(5:0).
	CS07	AAABAAABAB	AAABAAAAAB	Ratios of AA:AB progeny in pods 480 (5:0), 484 (4:0).

Table A2-1. Reassigned maternal inferred genotypes of *Plumeria rubra.* (continued)

Population	**Family**	**Inferred maternal multilocus genotype**	**Modified maternal multilocus genotype**	**Justification for modification**
Sandillal	00	AABBABAAAB	AABBAAAAAB	Ratios of AA:AB progeny in pods 305 (5:0), 229 (4:0), 300 (5:0)
	03	ABBBAAAAAA	AABBAAAAAA	Ratio of AA:AB progeny in pod 318 (7:0).

Table A3-1. Tree species of Hacienda La Pacífica, Guanacaste province, Costa Rica, classified according to breeding system and self-compatibility

Breeding System Family	Compatibility		
	Self-incompatible	Self compatible	No data
Hermaphroditic			
Annonaceae	*Sapranthus palanga*[1]		*Annona purpurea*, *A. reticulata*
Apocynaceae	*Plumeria rubra*		*Stemmadenia obovata*, *Thevetia ovata*
Araliaceae			*Sciadodendron excelsum*
Bignoniaceae	*Godmania aesculifolia*[1], *Tabebuia ochraceae*[1], *T. rosea*[1]		*Crescentia alata*, *Tabebuia impetiginosa*
Boraginaceae	*Cordia alliodora*		*Cordia bicolor*
Bombacaceae	*Bombacopsis quinata*, *Ochroma pyrimidale*[1]	*Ceiba pentandra*[2]	*Ceiba aesculifolia*, *Pseudobombax septenatum*
Capparidaceae			*Capparis frondosa*, *C. incana*
Celastraceae			*Maytenus segoviarum*
Chrysobalanaceae			*Licania arborea*
Cochlospermaceae	*Cochlospermum vitifolium*[1]		
Dillinaceae		*Curatella americana*[1]	
Elaeocarpaceae		*Muntingia calabura*[1], *Sloanea terniflora*[1]	

[1]Bawa, 1974; [2]Murawski and Hamrick, cited in Nason and Hamrick, 1997; [3]heterostylous (Bullock, 1985), assumed self-incompatible; [4]Bullock, 1985; [5]Bawa *et al.* 1985a;[6] James *et al.* 1998; [7]Loveless and Gullison, 2002; [8]Nason and Hamrick, 1997; [9]Murawski and Hamrick, 1991;. [10]Bawa and Opler 1975; [11]Witsberger *et al.* 1982;[12]Bawa and Webb, 1984

Table A3-1. Tree species of Hacienda La Pacífica, Guanacaste province, Costa Rica, classified according to breeding system and self-compatibility (continued)

	Compatibility		
Breeding System Family	Self-incompatible	Self compatible	No data
Hermaphroditic			
Erythroxylaceae	*Erythroxylon havanense*[3]		
Flacourtiaceae	*Casearia corymbosa*[4], *C. tremula*[4]	*Prockia crucis*[4]	*Casearia aculeata*
Lauraceae			*Ocotea veraguensis*
Leguminosae			
Caesalpinoideae	*Hymenaea courbaril*[1]		*Cassia emarginata, C. grandis, Schizolobium parahybum, Swartzia cubensis*
Mimosoideae	*Enterolobium cyclocarpum*[1], *Pithecellobium saman*[1]		*Acacia farnesiana, Albizia adinocephala, A. caribaea, A. guachepele, Inga vera, Lysiloma desmostachys, L. divaricatum, Pithecellobium longifolium,*
Papilinoideae	*Andira inermis*[1], *Dalbergia retusa*[1], *Lonchocarpus costaricensis*[1], *L. eriocarinalis*[1], *Myrospermum frutescens*[12], *Piscidia carthagenensis*[1], *Pterocarpus rohrii*[1]		*Acosmium panamense, Diphysa robinoides, Lonchocarpus minimiflorus, Machaerium biovulatum, Platymiscium pleiostachys, Willardia schiedeana*

[1] Bawa, 1974; [2] Murawski and Hamrick, cited in Nason and Hamrick, 1997; [3] heterostylous (Bullock, 1985), assumed self-incompatible; [4] Bullock, 1985; [5] Bawa *et al.* 1985;[6] James *et al.* 1998; [7] Loveless and Gullison, 2002; [8] Nason and Hamrick, 1997; [9] Murawski and Hamrick, 1991;. [10] Bawa and Opler 1975; [11] Witsberger *et al.* 1982;[12] Bawa and Webb, 1984

Table A3-1. Tree species of Hacienda La Pacífica, Guanacaste province, Costa Rica, classified according to breeding system and self-compatibility (continued)

	Compatibility		
Breeding System Family	Self-incompatible	Self compatible	No data
Hermaphrodite			
Malpighiaceae		*Byrsonima crassifolia*[1], *Malpighia glabra*[1]	
Myrsinaceae		*Ardisia revoluta*[1]	
Rubiaceae	*Hamelia patens*[5]	*Calycophyllum candidissimum*[1]	*Exostema mexicanum, Coutarea hexandra, Guettarda macrosperma*
Rutiaceae			*Esenbeckia litoralis*
Sapotaceae			*Manilkara chicle, Mastichodendron capiri*
Sterculiaceae	*Guazuma tomentosa*[1]		*Sterculia apetala*
Styracaceae			*Styrax argenteus*
Tiliaceae	*Luehea candida*[1], *L. speciosa*[1]		*Apeiba tiborbou,*
Verbenaceae			*Rehdera trinervis*
Monoecious			
Anacardiaceae	*Spondias mombin*[1]		
Euphorbiaceae			*Hura crepitans, Croton niveus*
Meliaceae	*Cedrela odorata*[6]; *Swietenia macrophylla*[7];		

[1]Bawa, 1974; [2]Murawski and Hamrick, cited in Nason and Hamrick, 1997; [3]heterostylous (Bullock, 1985), assumed self-incompatible; [4]Bullock, 1985; [5]Bawa *et al.* 1985;[6] James *et al.* 1998; [7]Loveless and Gullison, 2002; [8]Nason and Hamrick, 1997; [9]Murawski and Hamrick, 1991;. [10]Bawa and Opler 1975; [11]Witsberger *et al.* 1982;[12]Bawa and Webb, 1984

Table A3-1. Tree species of Hacienda La Pacífica, Guanacaste province, Costa Rica, classified according to breeding system and self-compatibility (continued)

	Compatibility		
Breeding System Family	Self-incompatible	Self compatible	No data / Not applicable (dioecious)
Monoecious			
Ulmaceae			*Trema micrantha*
Moraceae	*Ficus hondurensis*[8], *F. elastica*[8], *F. insipida*[8], *F. ovalis*[8]		
Sapindaceae			*Thouinidium decandrum*
Andromonoecious			
Anacardiaceae		*Anacardium excelsum*	
Leguminosae			
Caesalpinoideae	*Caesalpinia eriostachys*[1]		
Gynodioecious			
Moraceae		*Brosimum alicastrum*[9]	
Dioecious			
Anacardiaceae			*Astronium graveolens*[10]
Boraginaceae			*Cordia collococca*[10]
Burseraceae			*Bursera simaruba*[10], *B. tomentosa*[10]
Ebenaceae			*Dyospiros nicaraguensis*
Euphorbiaceae			*Bernardia nicaraguensis*[10]

[1]Bawa, 1974; [2]Murawski and Hamrick, cited in Nason and Hamrick, 1997; [3]heterostylous (Bullock, 1985), assumed self-incompatible; [4]Bullock, 1985; [5]Bawa *et al.* 1985;[6] James *et al.* 1998; [7]Loveless and Gullison, 2002; [8]Nason and Hamrick, 1997; [9]Murawski and Hamrick, 1991;. [10]Bawa and Opler 1975; [11]Witsberger *et al.* 1982;[12]Bawa and Webb, 1984

Table A3-1. Tree species of Hacienda La Pacífica, Guanacaste province, Costa Rica, classified according to breeding system and self-compatibility (continued)

	Compatibility		
Breeding System Family	Self-incompatible	Self compatible	Not applicable (dioecious)
Dioecious			
Meliaceae			*Guarea excelsa*[10], *Trichilia americana*[4]; T. *hirta*[10],T: *martiana*
Menispermaceae			*Hyperbaena tonduzii*[11]
Moraceae			*Chlorophora tinctorea*[10], *Cecropia peltata*[10], *Trophis racemosa*[2]
Polygonaceae			*Coccoloba caracasana*[10]
R[illegible]iaceae			*Genipa americana*[5]
[illegible]indaceae			*Allophyllus occidentalis10*
Simaroubaceae			*Alvaradoa amorphoides10, Picramnia latifolia10, Quassia amara, Simarouba glauca10*

[1]Bawa, 1974; [2]Murawski and Hamrick, cited in Nason and Hamrick, 1997; [3]heterostylous (Bullock, 1985), assumed self-incompatible; [4]Bullock, 1985; [5]Bawa *et al.* 1985;[6] James *et al.* 1998; [7]Loveless and Gullison, 2002; [8]Nason and Hamrick, 1997; [9]Murawski and Hamrick, 1991;. [10]Bawa and Opler 1975; [11]Witsberger *et al.* 1982;[12]Bawa and Webb, 1984

Table A3-1. Tree species of Hacienda La Pacífica, Guanacaste province, Costa Rica, classified according to breeding system and self-compatibility (continued)

	Compatibility		
Breeding System Family	Self-incompatible	Self compatible	Not applicable (dioecious)
dioecious			
Sapindaceae			*Allophyllus occidentalis*[10]
Simaroubaceae			*Alvaradoa amorphoides*[10], *Picramnia latifolia*[10], *Quassia amara*, *Simarouba glauca*[10]

[1]Bawa, 1974; [2]Murawski and Hamrick, cited in Nason and Hamrick, 1997; [3]heterostylous (Bullock, 1985), assumed self-incompatible; [4]Bullock, 1985; [5]Bawa *et al.* 1985;[6] James *et al.* 1998; [7]Loveless and Gullison, 2002; [8]Nason and Hamrick, 1997; [9]Murawski and Hamrick, 1991;. [10]Bawa and Opler 1975; [11]Witsberger *et al.* 1982;[12]Bawa and Webb, 1984

Table A3-2. Tree species of Hacienda La Pacífica, Cañas, Guanacaste, Costa Rica, classified by pollinator type and diaspore dispersal vector

	Diaspore dispersal vector[1]	
	Biotic	Abiotic or no apparent dispersal mechanism
Pollen vector[1]		
Bats		
Bombacaceae		*Bombacopsis quinata*[2] *Ceiba aesculifolia*[2] *C. pentandra*[2], *Ochroma lagopus*[2], *Pseudobombax septenatum*[2]
Bignoniaceae	*Crescentia alata*[3]	
Leguminosae		
Caesalpinoidea	*Hymenea courbaril*[4]	
Mimisoideae	*Inga vera*[5]	
Sapotaceae	*Manilkara chicle*[6]	
Hummingbirds		
Rubiaceae	*Hamelia patens*[7]	
Simaroubaceae	*Quassia amara*[8]	
Hawkmoths		
Apocynaceae		*Plumeria rubra*[9]
Bombacaceae		*B. quinata*[2]
Leguminosae		
Mimisoidea	*Enterolobium cyclocarpum*[10], *Inga vera*[5], *Pithecellobium saman*[11]	*A. guachepele*[11], *Pithecellobium longifolium*[12] (water?)
Rubiaeae	*Guettarda macrosperma*[13]	*Coutarea hexandra*[14]
Tiliaceae		*Luehea candida*[11], *L. speciosa*[11]
Medium-to-large bees		
Apocynaceae	*Stemmadenia obovata*[15], *Thevetia ovata*[16]	

Table A3-2. Tree species of Hacienda La Pacífica, Cañas, Guanacaste, Costa Rica, classified by pollinator type and diaspore dispersal vector (continued)

	Diaspore dispersal vector[2] Biotic	Abiotic or no apparent dispersal mechanism	No data
Pollen vector[1]			
Bignoniaceae		*Godmania aesculifolia*[17], *Tabebuia impetiginosa*[18], *T. ochraceae*[18], *T. rosea*[18]	
Cochlospermaceae		*Cochlospermum vitifolium*[19]	
Dilleniaceae	*Curatella americana*[20]		
Leguminosae			
Caesalpinoidea	*Cassia grandis*[21], *Swartzia cubensis*[22]	*Caesalpinia eriostachys*[23], *Schizolobium parahybum*[24]	*Cassia emarginata*
Papilionoideae	*Andira imermis*[25]	*Dalbergia retusa*[26] *Lonchocarpus costaricensis*[27], *L. eriocarinalis*[28], *Platimyscium pleiostachymum*[29], *Pterocarpus rohrii*[28], *Myrospermum frutescens*[28], *Piscidia carthaginensis*[28]	
Malpighiaceae	*Byrsonima crassifolia*[30], *Malpighia glabra*[31]		
Medium-large bees			
Rubiaceae	*Genipa americana*[28]		
Styracaeae	*Styrax argenteus*[32]		
Tiliaceae	*Apeiba tiborbou*[33]		

Table A3-2. Tree species of Hacienda La Pacífica, Cañas, Guanacaste, Costa Rica, classified by pollinator type and diaspore dispersal vector (continued)

	Diaspore dispersal vector[2]	
	Biotic	Abiotic or no apparent dispersal mechanism
Pollen vector[1]		
Small bees, moths, small diverse insects		
Anacardiaceae	*Anacardium excelsum*[34], *Spondias mombin*[35]	*Astronium graveolens*[36]
Araliaceae	*Sciadodendron excelsum*[37]	
Boraginaceae	*Cordia bicolor*[38], *C. colococca*[39]	*Cordia alliodora*[40]
Burseraceae	*B. simaruba*[41], *B. tomentosa*[42]	
Celastraceae	*Maytenus segoviarum*[43]	
Chrysobalanaceae	*Licanea arborea*[44]	
Ebenaceae	*Dyospiros nicaraguensis*[45]	
Eleocarpaceae	*Muntingia calabura*[44], *Sloanea terniflora*[46]	
Flacourtiaceae	*Casearia aculeata*[47], *C. corymbosa*[48], *C. tremula*[47], *P. crucis*[49]	
Lauraceae	*Ocotea veraguensis*[50]	
Leguminosae		
Mimosoidea	*Acacia farnesiana*[51]	
Papilionoidea		*Acosmium panamense*[52], *Lonchocarpus minimiflorus*[53], *Machaerium biovulatum*[47]
Meliaceae	*Guarea excelsa*[54], *Trichilia hirta*[55], *T. americana*[55], *T. martiana*[55]	*Cedrela odorata*[56], *Swietenia macrophylla*[57]
Menispermaceae	*Hyberbaena tonduzii*[58]	
Moraceae	*Brosimum alicastrum*[59], *Chlorophora tinctorea*[60]	

Table A3-2. Tree species of Hacienda La Pacífica, Cañas, Guanacaste, Costa Rica, classified by pollinator type and diaspore dispersal vector (continued)

	Diaspore dispersal vector[2]	
	Biotic	Abiotic or no apparent dispersal mechanism
Pollen vector[1]		
Small bees, moths, small diverse insects		
Myrsinaceae	*Ardisia revoluta*[61]	
Polygonaceae	*Coccoloba caracasana*[62]	
Rubiaceae		*Calycophyllum candidissimum*[63]
Rutaceae		*Esenbeckia litoralis*[64]
Small bees, moths, small diverse insects		
Sapindaceae	*Allophyllus occidentalis*[65]	*Thouinidium decandrum*[47]
Sapotaceae	*Mastichodendron capiri*[65]	
Simaroubaceae	*Picramnia latifolia*[66], *Simarouba glauca*[67]	*Alvaradoa amorphoides*[47]
Sterculiaceae	*Guazuma ulmifolia*[68],	
Ulmaceae	*Trema micrantha*[69]	
Pollen vector[1]		
Beetles		
Annonaceae	*Saprantbus palanga*[70] *Annona purpurea*[71], *A. reticulata*[71]	
Wind		
Moraceae	*Cecropia peltata*[72], *Trophis racemosa*[73]	
Fig wasps	*Ficus elastica, F. bondurensis, F. insipida, F. ovalis*	

Table A3-2. Tree species of Hacienda La Pacífica, Cañas, Guanacaste, Costa Rica, classified by pollinator type and diaspore dispersal vector (continued)

	Diaspore dispersal vector[2]		
	Biotic	Abiotic or no apparent dispersal mechanism	No data
Pollen vector[1]			
Not classified	*Capparis frondosa*[74], *C. incana*[74]; *Sterculia apetala*[75]	*Albizia caribaea*, *A. adinocephala*; *Exostemma mexicanum*, *Hura crepitans*, *Lysiloma divaricatum*[76]; *L. desmostyachys*[77] *Rehdera trinervis*[78]	*Diphysa robinoides*, *Erythroxylon havanense*, *Bernarida nicaraguensis*, *Croton niveus*, *Willardia schiedeana*

[1] Sources/justification: see footnote for each entry; [2] PV (pollen vector): Heithaus *et al.*, 1975, DV (diaspore vector): wind (kapok 'floaters'); [3] PV: Heithaus *et al.*, 1975, DV: Janzen, 1983b); [4] PV: Heithaus *et al.*, 1975, DV: Janzen, 1983c; [5] PV: Heithaus *et al.*, 1975, Koptur, 1983; [6] Heithaus *et al.*, 1975; [7] PV:Bawa et al., 1985b, DV: juicy berry (Salas Estrada, 1993); [8] PV: Janzen and Liesner, 1980, DV: fleshy drupe, Standley and Steyermark, 1946a; [9] PV: Haber and Frankie, 1989, DV: Haber, 1984; [10] PV: Apsit *et al.*, 2001, DV: Janzen and Martin, 1982; [11] PV: Haber and Frankie, 1989, DV: Janzen and Liesner, 1980; [12] PV:Haber and Frankie, 1989, DV: Frankie *et al.*, 1974; [13] PV: Haber and Frankie, 1989, DV: fleshy fruit, Enquist and Sullivan, 2001; [14] PV: Haber and Frankie, 1989, DV: winged seed, Witsberger *et al.*, 1982; [15] PV: Frankie *et al.*, 1983, DV: Foster and McDiarmid, 1983; [16] PV: Frankie *et al.*, 1983, DV: fleshy fruit, Janzen and Liesner, 1980; [17] PV: Frankie *et al.*, 1983, DV: Witsbeger et al., 1982; [18] PV: Frankie *et al.*, 1983, DV: Gentry, 1983; [19] PV: Frankie *et al.*, 1983, DV: Bawa and Frankie, 1983; [20] PV: Frankie *et al.*, 1983, DV: Janzen, 1967, Salas Estrada, 1993; [21] PV:Frankie *et al.*, 1983, DV: 'molasses' in pods; [22] PV: Bawa et al., 1985b, DV: arillate seed (Janzen and Leisner, 1980); [23] PV: Frankie et al., 1983, DV: explosively dehiscent pod, Bawa and Webb, 1984; [24] PV: large, yellow flowers, DV: winged seed; [25] PV: Frankie *et al.*, 1983, DV: Heithaus *et al.*, 1975; [26] PV: Frankie et al., 1983, DV: wind, Bawa and Webb, 1984; [27] PV: Frankie et al., 1983, DV: wind dispersal appears to be generalized in this genus, *e.g.* congeners listed in Augsberger (1986), Ibarra-Manríquez, 2001; [28] PV: Frankie et al., 1983, DV: Janzen and Liesner, 1980; [29] PV: Frankie et al., 1983, DV: based on similarity to wind-dispersed congener *P. dimorphandrum* (Witsberger et al., 1982); [30] PV: Frankie et al., 1983, DV: Anderson, 1983; [31] PV: 'oil-flowers' (Gottsberger, 1986), DV: birds (Anderson, 1983); [32] PV: Frankie et al., 1983, DV: Camacho and Orozco, 1998; [33] PV: Frankie et al., 1983, DV: Janzen and Martin, 1982; [34] PV: Ghazoul and McLeish, 2001, Heithaus *et al.*, 1975; [35] PV: Nason and Hamrick, 1997, Heithaus *et al.*, 1975; [36] PV: small flowers, DV: wind (Augsbeger, 1986);[37] PV: small flowers, DV: 'blueberry-like' fruits (Endquist and Sullivan, 2001); [38] PV: small flowers, DV: fleshy fruit (Zamora, 2000); [39] PV: small flowers, DV: fleshy fruit (Little *et al.*, 1974); [40] PV: moths (Bawa et al., 1985b), DV: Janzen and Liesner, 1980; [41] PV: small diverse insects (Bawa et al., 1985b), DV: Stevens, 1983; [42] PV: based on similarity to *B. simaruba*, DV: fleshy fruit (Janzen and Liesner, 1980); [43] PV, DV: Enquuist and Sullivan, 2001; [44] PV: small flowers, DV: Heithaus *et al.*, 1975; [45] PV: small flowers, DV: Janzen and Martin, 1982; [46] PV: small flowers, DV: Elizondo, 1999; [47] PV: small flowers, DV: Janzen and Liesner, 1980; [48] PV: small flowers, DV : Janzen, 1983d; 49PV: small flowers, DV: fleshy fruit, Endquist and Sullivan, 2001; [50] PV: small flowers, DV : Masís *et al.*, 1998; [51] PV: small, fragrant flowers, DV: Janzen and Martin, 1982; [52] PV: small flowers, DV: wind (Molina, 1996); [53] PV: small flowers, DV: Enquuist and Sullivan, 2001; [54] PV: PV: moths, based on congeners listed by Bawa *et al.*, 1985b, DV: arillate seeds (Standley and Steyermark, 1946a; [55] PV: small flowers, DV: arillate seeds, Enquuist and Sullivan, 2001; [56] PV: moths (Bawa *et al.*, 1985b), DV: Janzen and Liesner, 1980; [57] PV: White *et al.*, 1999; DV: Janzen and Liesner, 1980; [58] Witsberger et al., 1982; [59] PV: Bawa *et al.*, 1985b, DV: Heithaus *et al.*, 1975; [60] PV: According to Bawa *et al.* (1985b), may be anemophilous, DV: Heithaus *et al.*, 1975; [61] PV: small flowers, DV: Enquuist and Sullivan, 2001; [62] PV: small flowers, DV: Foster, 1983; [63] PV: small flowers, DV: Janzen, 1967; [64] PV: small flowers, DV: explosive capsule; [65] PV: small flowers, DV: fleshy fruit (Janzen and Liesner, 1980); [66] PV: small flowers,DV: baccate fruit (Standley and Steyermark, 1946a); [67] PV: Bawa *et al.*, 1985b, DV: fleshy fruit (Janzen and Liesner, 1980); [68] PV: small flowers, DV: Janzen, 1983e; [69] PV: small flowers, DV: Hartshorn, 1983; [70] PV, DV: Janzen, 1983a; [71] PV: beetle pollinated genus; see congeners in Bawa *et al.*, 1985b; DV: Janzen and Martin, 1982; [72] PV: wind (Janzen, 1967), DV: Heithaus *et al.*, 1975; [73] PV: Bawa *et al.* (1985b), DV: fleshy fruit (Janzen and Liesner, 1980); [74] DV: fleshy fruit (Little *et al.*, 1974); [75] DV: Janzen, 1967; [76] DV: based on similarity with *L. desmostachys*; [77] DV: Janzen and Liesner, 1980; [78] DV: wind (Molina, 1996);

Table A3-3. Population densities of tree species of Hacienda La Pacífica, Cañas, Guanacaste, Costa Rica (data based on Glander and Nisbett, 1996; Jiménez et al., 1987)

Species	Density (stems ≥ 1cm dbh ha^{-1}	
	Riparian[1]	Upland[2]
Anacardiaceae		
Anacardium excelsum	29	0
Astronium graveolens	6.75	8.3
Spondias mombin	4.15	0.2
Annonaceae		
Annona purpurea	1.4	0.6
Annona reticulata	2.35	2.1
Sapranthus palanga	0.2	3.7
Apocynaceae		
Plumeria rubra	0.1	0
Stemmadenia obovata	2.7	6
Thevetia ovata	1.3	0.2
Araliaceae		
Sciadodendron exclesum	0.1	0.2
Bignoniaceae		
Crescentia alata	0	0
Godmania aesculifolia	0	1.4
Tabebuia impetiginosa	0.1	1.9
Tabebuia ochraceae	6.95	52.4
Tabebuia rosea	4.35	0.4
Bombacaceae		
Bombacopsis quinata	1	1.7
Ceiba aesculifolia	0	0
Ceiba pentandra	0.8	0
Ochroma lagopus	0.1	0
Pseudobombax septenatum	0.1	0
Boraginaceae		
Cordia alliodora	9.25	68.4
Cordia bicolor	1.6	9.1
Cordia colococca	7.7	28.9
Burseraceae		
Bursera simaruba	2.5	1.2
Bursera tomentosa	0	0
Caesalpinaceae		
Caesalpinia eriostachys	0	0
Cassia emarginata	0.4	5
Cassia grandis	0.9	0
Hymenaea courbaril	7.4	1.9
Schizolobium parahybum	1.1	0
Swartzia cubensis	0	0

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[1] Mean of Glander and Nisbett's two riparian areas; total riparian area: 9.91ha;

[2] Area of upland area: 4.81ha

Table A3-3. Population densities of tree species of Hacienda La Pacífica, Cañas, Guanacaste, Costa Rica (data based on Glander and Nisbett, 1996; Jiménez et al., 1987) (continued)

Species	Density (stems ≥ 1cm dbh ha^{-1}	
	Riparian[1]	Upland[2]
Capparidaceae		
Capparis frondosa	0	0
Capparis incana	0.1	0
Celastraceae		
Maytenus segoviarum	0	0
Chrysobalanaceae		
Licania arborea	5	0.4
Cochlospermaceae		
Cochlospermum vitifolium	1	1
Dillenaceae		
Curatella americana	0	0
Ebenaceae		
Dyospiros nicaraguensis	7.75	8.9
Eleocarpaceae		
Muntingia calabura	3.6	0.8
Sloanea terniflora	2.6	0.2
Erythroxylaceae		
Erythroxylon havanense	0	0
Euphorbiaceae		
Bernardia nicaraguensis	0	0
Croton niveus	0	0
Hura crepitans	0.2	0
Flacourtiaceae		
Casearia aculeata	2.3	8.5
Casearia corymbosa	2.5	11.6
Casearia tremula	0.1	0
Prockia crucis	0	0
Lauraceae		
Ocotea veraguensis	0	0
Malpighiaceae		
Byrsonima crassifolia	0.5	0.2
Malpighia glabra	0.2	0
Meliaceae		
Cedrela odorata	1	0
Guarea excelsa	0	0
Swietenia macrophylla	12.05	5.6
Trichilia hirta	0	0
Trichilia americana	3.9	12.3
Trichilia martiana	1.95	0.2

[1] Mean of Glander and Nisbett's two riparian areas; total riparian area: 9.91ha;

[2] Area of upland area: 4.81ha

Table A3-3. Population densities of tree species of Hacienda La Pacífica, Cañas, Guanacaste, Costa Rica (data based on Glander and Nisbett, 1996; Jiménez *et al.*, 1987) (continued)

Species	**Density (stems ≥ 1cm dbh ha^{-1}**	
	Riparian[1]	Upland[2]
Menispermaceae		
Hyberbaena tonduzii	0	0
Mimisoideae		
Acacia farnesiana	0	0
Albizia adinocephala	0.3	3.7
Albizia caribaea	1.7	25.8
Albizia guachepele	4.7	1.9
Enterolobium cyclocarpum	4.6	3.1
Inga vera	1.4	0
Lysiloma desmostachys	0	0
Lysiloma divaricatum	3.45	29.1
Pithecellobium longifolium	4.65	0
Pithecellobium saman	2.5	0.6
Moraceae		
Brosimum alicastrum	0	0
Cecropia peltata	0.2	0
Chlorophora tinctorea	1.5	3.3
Ficus hondurensis	0	0
Ficus elastica	0	0
Ficus insipida	0	0
Ficus ovalis	0.5	0.42
Trophis racemosa	0	0
Myrsinaceae		
Ardisia revoluta	4.8	0
Papilionoideae		
Acosmium panamense	0.4	0.6
Andira inermis	2.65	0
Dalbergia retusa	4.3	4
Diphysa robinoides	0	0
Lonchocarpus costaricensis	0	0.4
Lonchocarpus eriocarinalis	0	0
Lonchocarpus minimiflorus	31.6	546
Machaerium biovulatum	2.3	11
Myrospermum frutescens	11.75	19.3
Piscidia carthagenesis	4.2	6
Platimiiscium pleostachyum	0.1	0.8
Pterocarpus rohrii	0	6.2
Willardia schiedeana	0	0

[1] Mean of Glander and Nisbett's two riparian areas; total riparian area: 9.91ha;
[2] Area of upland area: 4.81ha

Table A3-3. Population densities of tree species of Hacienda La Pacífica, Cañas, Guanacaste, Costa Rica (data based on Glander and Nisbett, 1996; Jiménez *et al.*, 1987) (continued)

Species	**Density (stems ≥ 1cm dbh ha^{-1}**	
	Riparian[1]	Upland[2]
Polygonaceae		
Coccoloba caracasana	2.3	0
Rubiaceae		
Calycophyllum candidissimum	4.35	3.7
Coutarea hexandra	0	0
Exostema mexicanum	0	0
Genipa americana	3.45	2.7
Guettarda macrosperma	0	0
Hamelia patens	0	0
Rutaceae		
Esenbeckia litoralis	0	0
Sapindaceae		
Allophylus occidentalis	1.3	4.1
Thouinidium decandrum	4.15	7.3
Sapotaceae		
Manilkara chicle	5.15	0
Mastichodendron capiri	0.6	1.2
Simaroubaceae		
Alvaradoa amorphoides	0	0
Picramnia latifolia	0	0
Quassia amara	0.2	0
Simarouba glauca	2.75	0.8
Sterculiaceae		
Guazuma tomentosa	60.35	68
Stercula apetala	0.7	0.2
Styracaceae		
Styrax argenteus	0	0
Tiliaceae		
Apeiba tiborbou	0.4	0
Luehea candida	8.8	23.7
Luehea speciosa	0.1	0.4
Ulmaceae		
Trema micrantha	0.6	0.6
Verbenaceae		
Rehdera trinervis	0.305	0
MEAN	2.81	8.93
MEDIAN	0.50	0.20

[1] Mean of Glander and Nisbett's two riparian areas; total riparian area: 9.91ha;
[2] Area of upland area: 4.81ha

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APPENDIX 4: LETTER OF PERMISSION FOR USE OF COPYRIGHT MATERIAL (OVERLEAF)